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NO. 40

STUDIES ON THE TOBACCO STUNT DISEASE

JUNE, 1956

HATANO TOBACCO EXPERIMENT STATION

HATANO, KANAGAWA, JAPAN

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Studies on the Tobacco Stunt Disease

Hatano Tobacco Experiment Station,

Hatano, Kanagawa,

Japan

STUDIES ON THE TOBACCO STUNT DISEASE

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Symptoms of the Tobacco Stunt Disease

EXPLANATION OF PLATE I

Fig. 1. Symptoms on the infected seedling.

Towards the base of lower leaves, necrotic spot, first brown and then white, are seen. Due to the necrosis of veins, leaves are conspicuously deformed, though top leaves are nearly normal.

Fig. 2. Symptoms on the infected seedling.

Top leaves are changed to yellow and are rolled downward from the tip of the leaves. In lower leaves necrosis is seen together with rolling and unevenness of the leaves.



Fig. 1



Fig. 2

EXPLANATION OF PLATE II

Fig. 1. Symptoms on the infected seedling.

Necrotic spots are seen scatteringly on the surface of the leaves. They are usually apparent when the sun-shine is strong. The whole leaf is rolled downward and is uneven. The top leaves are changed yellowish white and necrosis is seen in veins.

Fig. 2. Symptoms of the infected plant in the field.

On lower leaves necrotic spots are seen in the shape of a ring, leaves are uneven and the internodes are ill-grown, i.e., the whole plant is stunted.

PL. II



Fig. 1



Fig. 2

EXPLANATION OF PLATE III

Fig. 1. Symptoms of the infected plant in the field.
On the top of the plant there are many deformed leaves, and the growth is remarkably hampered. Due to ill-growth of the internodes it is apparently "stunted".

Fig. 2. Symptoms of the infected plants in the field.
Both healthy and infected plants are seen, showing the difference of growth before topping time. Four ridges in the front show diseased plants, and four ridges in the back show healthy plants.



Fig. 1



Fig. 2

HIDAKA, Z. & NAKANO, K. (1956). *Bull. Hatano Tobacco Expt. Sta. 40, 1–12.*

STUDIES ON THE TOBACCO STUNT DISEASE

I. RESULTS OF THE RESEARCH PARTY ON THE TOBACCO STUNT DISEASE

By Zyun HIDAKA and Koretoshi NAKANO

INTRODUCTION

When a new disease is found it is most important that a control measure is worked out at earliest possible date. On the disease to be reported here, a plan was made as early as the latter part of 1948, in forming a research and investigation party consisting of all the members who were engaged in research work of tobacco disease and insect belong to the Japan Monopoly Corporation. This party was organized during the period January to May 1949, and was equiped with faculty to perform all kinds of experiments which could be thought of at that time. The Takehara District of the Corporation (Kamo-gun, Hiroshima Prefecture) where this disease was most frequently reported was chosen as the site of study.

This report is a result of the cooperation of all participating members of the party. The writers only take the responsibilities of the work. The participating members were as follows:

Hatana Tobacco Experiment Station: Zyun HIDAKA, Toshifumi TORII, Ichiro TAKAOKA, Tadao SHIMIZU, Kiyoshi KIRIYAMA, and Tsutomu SEKIYA (with the help of Toshiharu NAKAMURA of the same station).

Okayama Tobacco Experiment Station: Koretoshi NAKANO, Yoshio OTANI, Shunichi OSHIMA, and Yukiharu KASHIWARA, (with the help of Mitsushige OKUYAMA and Sanzo FURUYA of the same station).

Kagoshima Tobacco Experiment Station: Hikotoshi TSUMAGARI, Isamu TANAKA, and Toshiro HONDA.

We express our gratitude to Mr. Chuji KAMBAYASHI, ex Director of Tobacco Production Department of the Japan Monopoly Corporation for providing budget needed in financing the research project work and also to Mr. Futoshi HOSHIKO, Hiroshima Regional Bureau of the Corporation, Rokuichi MURAKAMI, Hiroshi KAWAGUCHI, Hitoo WAKIMOTO, Takaji IIDA, Kenichi TAKAHASHI, and Isao WAKISAKA of Takehara Office of the Corporation, who assisted us in our work. We also express our thanks to Prof. Dr.

Hajime YOSHII for his personal guidance in the research area. Cooperation is acknowledged to many others, especially to those owners of the experimental seedbeds.

HISTORY OF THE DISEASE AND MOTIVES FOR RESEARCH

The earliest record of the present disease goes back as early as 1931, when seedlings in 180 *tsubo* seedbeds (about 6 are) at Tadanoumi-cho, Toyoda-gun, Hiroshima Prefecture, were almost completely destroyed by this disease. Since then this disease has been called in this locality "Tadanoumi Disease". Cultivators and instructors of tobacco cultivation in the area remember details of the circumstances. Since then, this disease was frequently reported in this area. In June 1935, Messrs. Kakugoro NAKATA and Seito TAKIMOTO, upon the request of Hiroshima Regional Bureau of the Japan Monopoly Corporation, investigated the tobacco root rot disease and brought back to the Phytopathological Laboratory of Kyushu University three tobacco plants which showed symptoms similar to this disease on the leaves and stems but none on the roots. The pictures of these plants are still kept in the laboratory. Grafting and sap inoculation were experimented on to healthy plants, but with no apparent results. In July 1939 HIDAKA, one of the writers, collected two samples on his way from Takehara-cho, Kamo-gun, Hiroshima Prefecture, to its adjacent village, Yoshina-mura, and another two at the Okayama Tobacco Experiment Station, and brought them back to the laboratory of Kyushu University. Experiments revealed appearance of some symptoms of the disease in the scion of the healthy plant grafted on the infected stock. In those days no serious damage was reported of this disease, and the disease was only rarely seen in the fields. The writer held interest in inoculating it as it was an unusual disease originating in the same way as "tobacco leafcurl". The symptoms of this disease being difficult to observe under high temperatures, the collections of the samples and the inoculation having been performed during summer months, it was presumed that no symptoms will appear under these conditions. In July 1941 he brought three more samples from the Okayama Tobacco Experiment Station to the laboratory. And in June 1942 two from Ayase-mura, Koza-gun, Kanagawa Prefecture, to the Hatano Tobacco Experiment Station for grafting and inoculation. Slight symptoms were discerned in autumn from these samples. At that time it was believed that the disease was transmitted by some insects in the same way as tobacco leaf curl. In June 1947 Messrs. HOSHIKO and MURAKAMI led HIDAKA, one of the writers, to Ato-mura, Kamo-gun, Hiroshima Prefecture, where it was quite surpris-

ing to see many fields of tobacco plants stunted 30 to 40 percent from the disease. In 1948 considerable damage was reported from Atsumi Peninsula, Aichi Prefecture, and one infected plant was found in this station and two at Hirasawa, Minamihatano-cho, Naka-gun, Kanagawa Prefecture. Additional samples were gathered at Aso-cho, Namekata-gun, Ibaraki Prefecture. From these facts that the disease has been spreading from the Seto Inland Sea Coast Area to various districts, urgent need of study was made apparent by HIDAKA, and NAKANO.

PRELIMINARY INVESTIGATION IN RESEARCH AREA

Special emphasis was placed on the preparatory study, as the disease in question was considered one absolutely new to the party, and the only possible idea that could be formed was that it must have been a kind of virus disease guessing from the condition in the field and the results of laboratory experience. All the instructors of tobacco cultivation belonging to the Takehara Office District (direct instructors employed by the Japan Monopoly Corporation), Mr. Shizuo MIYAGAWA who was on duty in the office at about 1931 and some enthusiastic tobacco cultivators were asked to be gathered to furnish the party with the informations on the development of the disease. From these investigations it was confirmed that the symptoms on the seedlings were apparent about 15 days before they were ready for transplanting, and that they became most conspicuous at the time of transplanting, resulting in seedling shortages. The stunt disease always resulted by planting these infected seedlings.

Controlling measures carried out in the area at that time were: (1) that new soil (virgin soil to crops) should be used as seedbed soil, (2) heat materials of seedbeds of the previous year should not be used as farmyard manure for the seedbed surface soil, and (3) steam sterilization and baking were partially utilized: but none of these measures showed definite effect. The fact was perhaps due to the reasons that the virus was carried by some vectors in the seedbed periods or that the soil was infected after its sterilization. In any way, since there was no definite measure the growers could only plant those seemingly healthy seedlings or purchase healthy seedlings from elswhere. Besides, the development of the disease was usually very uncertain, although there were in which the disease was serious consistently, as the serious damage of the previous year did not necessarily cause similar damage the next year. It is also presumed that it was not contagious in the field. Quite an amount of information was gathered, but very few facts were common to every experience nor even related with each other.

There were no report as to insect vector. It was learned that this disease was called by many different names, e.g., Tadanoumi-byo (mentioned above), Chijimi-byo (shrink disease), Ishuku-byo (dwarf disease), Izari-byo (cripple disease), Gazo-byo (crab disease), Hikkomi-byo (draw-back disease), and Sukumi-byo (cower disease).

Cultivators in Tono-mura, Kamo-gun, Hiroshima Prefecture, have been used to purchasing seedlings from its adjacent village Shono-mura to make up for the shortages in seedlings because of this disease, but in 1948 almost all the seedlings in Shono-mura were destroyed while in Tono-mura it was only seen in the same degree as in previous years.

Method of study and investigation

From the preparatory study, it was learned that the infection of this disease was already performed in the seedbed period. The plan was constructed to centralize investigation on the seedbeds as shown below:

Group for tracing infection: seedbed soil (sub-soil and surface soil) sterilization, seedbed material sterilization or by using new materials.

Group for researching vector: by collecting all the insects in seedbed period and by using insecticides.

Since our preparatory study showed that damage was reported annually from the same area but the conditions differred according to the section or among the seedbed, two seedbeds in Shono-mura where the most serious damage was recorded in the previous year, and three seedbeds in Tono-mura where the disease was reported every year set up. From their experience in the damage of this disease in 1947 and also in previous years the following seven people were asked as the keepers of the experimental seedbeds.

Kaichi TAKAHARA	Shono-mura,	Kamo-gun,	Hiroshima Prefecture
Yukio KARASAKI	”	”	”
Yoshio FUKUGAWA	Tono-mura,	”	”
Tokuo YAMAUCHI Matsuzo MORITA	”	”	”
Motozi SANETO Tokumi SANETO	”	”	”

The possible channels of spread of this disease in seedbeds were: (1) soil infection, (2) contamination of seedbed materials used in the previous year, i.e., seedbed cloth, piles, braces and wire, (3) contamination from heating materials, and (4) insects and other animals.

With these points in consideration, we decided to set up the following five sections in each experimental seedbed:—

Section 1. Surface soil and sub-soil sterilization, use of new seedbed materials, and heating of seedbed by electricity.

Section 2. Surface soil and sub-soil not sterilized, but use new seedbed materials, and heat seedbeds by electricity.

Section 3. Surface soil and sub-soil sterilization, but use old seedbed materials, and heat seedbed by electricity.

Section 4. Surface soil and sub-soil sterilization, use new seedbed materials, and ordinary raised seedbed.

Section 5. Surface soil and sub-soil not sterilized, use old seedbed materials, and ordinary raised seedbed (usual method).

Each section was 2-*tsubo* in size and was independent of each other. Surface soil sterilization was carried out with steam by holding over 70°C for 30 minutes. (The temperature was actually over 90°C or almost 100°C in most of the cases.) Seedbed sub-soil was mostly sterilized in the same way with steam but in some cases by baking it on an iron plate stirring it until it was white. It is presumed that the temperature of the soil reached at least 95°C. It took seven days to finish all the sterilization. Special care was taken the soil from contaminating. This was done by spreading insecticide (0.5 percent BHCγ powder), and injecting 10 ml of chloropicrin per 1 m^3 into the sterilized soil. It was hard to sterilize seedbed materials, so new materials were used taking every care not to contaminate them. As it was impossible to sterilize the heat materials in the ordinary raised seedbeds, electricity was used at the heat source. Materials used for this was also sterilized so that they looked white with BHCγ 0.5 percent powder or else by steam treatment. There was no definite order as to the arrangement of these sections. On two out of every five experimental areas, insecticides were used after the establishment of the seedbeds in order to prevent infection via insect vectors and other animals. During the establishment of the seedbeds when soils were being brought in utmost effort was made to prevent unsterilized soil and other materials from being mixed in.

Preparation of the establishment of the seedbeds was started on the 2nd February 1949, sterilization of surface soil and sub-soil on the 9th and finished the same on the 15th. The establishment of the seedbeds was finished on the 18th February, and seeds were sown on the 19th and 20th.

Air and soil temperatures were measured at 10:00 and 16:00 daily. A self-recording thermometer were used for air temperature. Chemical application was experimented on YAMAUCHI-MORITA and KARASAKI's seedbeds, and BHC wettable powder was applied every 5 to 7 days.

Around 23rd March, however, injury by the chemical was recognized and had to stop using BHC. After the nicotine sulphate 40 (1/800 in dilution) mixed with soap was used daily or every other day.

Members of the research party took turns so that two were stationed at experimental area. They inspected all seedbeds twice in the morning and twice in the afternoon, collecting insects and other animals and studying any indications, if any, of disease or other abnormalities. Transplanting in the field was done separately according to each section of the seedbeds to enable us to discern any symptoms of infection and observed transmission of the disease in the field.

RESULTS

The results of the experiments, according to each seedbed keeper, are as shown on Table 1. The number of total diseased plants according to the section is: Section 1, 21; Section 2, 1956; Section 3, 41; Section 4, 11; Section 5, 1032. It is hard to compare the results of YAMAUCHI-MORITA's seedbeds with others, because in their seedbeds the number of diseased plants was too small. In other four areas, however, there were many in Sections 2 and 5, where the sub-soil and surface soil were not sterilized. The diseased plants occurred scattered through out the bed and there was no indication that the infection was concentrated (see I-PL. II, Fig. 1). In Sections 1, 3 and 4, where the sub-soil and surface soil were sterilized, diseased plants were very few and mostly in groups (see I-PL. I, Fig. 2). Moreover they tended to develop around the border of the seedbeds. The 11th of April the first day the diseased plants were recognized. It was in FUKUKAWA's seedbed where there were several of them. On 13th two was found in SANETO-SANETO's seedbeds, on 14th several in TAKAHARA's and on 15th the infected plants were confirmed that they were the disease in question. On every diseased plant a piece of straw was placed to mark the spot.

After 15th of April we inspected very carefully each plant every day in all the seedbeds recording the number of the plants infected. After a rain, rather than on rainy days, more infected plants were discovered, perhaps due to the fact that after rain it was easier for to discern the symptoms. When the indication of the disease was first seen, the plant had about six leaves including cotyledons and would be ready for transplanting by at least 15 days. We transplanted the plants in more than sixty *tsubo* of the field, and after transplanting, the infected plants developed typical symptoms of the disease in question. We did not recognize any sign of infection in the field.

TABLE 1. *Number of Seedlings Infected with the Tobacco stunt Disease In 1949 Experiment*

		Dates of investigation											
		April 15	16	17	18	19	20	21	22	23	24	25	Total
Yamauchi	No. 1	0	0	0	0	0	0	0	0	1	0	0	1
Morita	〃 2	0	0	0	0	0	0	0	0	0	0	0	0
	〃 3	0	0	0	0	0	0	0	0	0	0	0	0
	〃 4	0	0	0	0	0	0	0	0	0	1	1	2
	〃 5	0	0	0	0	0	0	0	0	0	0	0	0
Saneto	No. 1	0	0	0	0	1	0	0	1	0	0	0	2
Saneto	〃 2	16	13	14	41	125	72	156	100	84	115	220	956
	〃 3	0	0	0	0	0	2	0	2	0	0	1	5
	〃 4	0	0	0	0	0	0	4	0	2	0	1	7
	〃 5	9	14	20	30	92	75	245	76	50	116	168	895
Fukukawa	No. 1	0	0	0	0	0	0	0	0	0	0	0	0
	〃 2	29	31	14	24	58	21	128	49	29	41	78	502
	〃 3	0	0	0	0	0	0	0	1	0	1	0	2
	〃 4	0	0	0	0	0	0	0	0	0	0	0	0
	〃 5	0	0	0	0	3	3	4	10	4	7	9	40
Karasaki	No. 1	0	0	0	0	0	0	1	0	0	0	0	1
	〃 2	2	2	0	4	3	12	12	6	9	9	—	59
	〃 3	0	0	0	0	0	0	0	2	0	1	0	3
	〃 4	0	0	0	0	0	0	0	0	0	0	0	0
	〃 5	0	2	0	4	5	8	13	9	7	15	32	95
Takahara	No. 1	0	0	2	1	1	4	2	5	0	2	0	17
	〃 2	6	3	0	12	8	6	3	3	2	0	0	43
	〃 3	0	3	3	2	0	13	4	1	1	4	0	31
	〃 4	0	0	0	0	0	0	1	0	0	0	1	2
	〃 5	0	0	1	0	0	0	1	0	0	0	0	2

Remarks: 1. Names given are those of the keepers.
2. Figures in the table indicate the number of diseased plants discovered on the dates specified.
3. Figures shown under April 15 indicate the total number of diseased plants discovered by the date.
4. Investigation was carried out from 15th to 30th of April, but after 25th it had to be suspended often because of transplanting. The results were, therefore, recorded up to 25th here, but there were some which showed the symptoms on the plants after transplanting in the field and on remaining seedlings in the seedbeds.

By the latter part of March, we collected *Onychiurus yagii* MIYOSHI, 3 *Stomoxys* species, *Tipula aino* ALEXANDER, *Tetranychus telarius* LINNE, *Tyroglyphus farinae* DE GEER, *Gryllotalpa africana* PALISOT DE

BEAUVOIS, the larva of *Nygmia subflava* BREMER, *Armadillidium vulgare* LATREILLE, *Hister jekeli* MARSEUL, *Aphodius rectus biformis* REITTER, *Panagaeus japonicus* CHAUDOIR, and *Xantholinus* spp. In the first part of April we collected some species of Ceratopogonidae, *Stomoxys* spp., the larva of *Nygmia subflava* BREMER, *Scarites pacificus* BATES, *Tetranychus lintetarius* LINNE, the larva of *Xantholinus* spp., *Paederus idae* LEWIS, *Rhizoglyphus echinopus* FUMOUZE et ROBIN, the larva of *Scarabaeids*, *Gryllotalpa africana* PALISOT DE BEAUVOIS, *Myzus persicae* SULZER. But these insects were not considered vectors for this disease.

These seemed to be a slight tendency for increased disease development when the moisture was excessive in the seedbed, but this was not considered a direct or too important reason. At a high soil moisture when the soil was sterilized there was no evidence of increased development of the disease. Neither was able to confirm with the rate of growth nor with the nutritional condition of the seedlings.

SYMPTOMS AND DISTRIBUTIONS OF THE DISEASE

At first, the angles of expanding leaves against the stem are greater than usual and the leaves tend to twist themselves. Toward the basal part of the leaves, veins develop veinclearing, and later the leaves sometimes show mottling. Expanded leaves first show veinlet clearing in line or in small spots and change to yellowish green, and then these infected parts indicate necrosis in spots. The width of the spot is about that of poppy-seed, and the spot never round but always with some irregular angles. The spot occurs also on veinlets and tends to spread in line. When these spots form a ring, the infected part spreads sometimes beyond the vein, while in the case of other tobacco disease it is always surrounded by the vein (see I-PL. I, Fig. 1). In this point somewhat it resembles symptoms of tobacco streak disease. The leaves of which the veins have developed vein-clearing soon grow dark green beginning with expanded leaves. This state lasts up to the end of the growth period in the field. When about the time the leaves become dark green, the internodes stop their growth, so that the diseased plants are hidden under healthy ones. They stand flat on the surface of the seedbed with the angle of the leaves against the stem greater than usual. The shape of the leaves, too, becomes slightly irregular, but it is not very conspicuous in lower leaves. The leaves are generally more or less narrower and smaller in size, and they grow as a rosette. The part of the infected plant level with the soil (3 to 5 mm below cotyledons, i. e., between the stem and the roots) becomes necrosis and strangulated. When

it is bent, it is easily broken at this point. Even if the plant looked healthy above ground, there were many cases where there was necrosis at the soil level. This fact is shown in Table 2.

72.2 percent of the plants seemingly healthy were strangulated. Of the total number, the percentage was as high as 77 percent. Both kinds were small in their seedling stages, and although we could not discern any indication of strangulation in these ill-grown seedlings, later investigation showed that once an indication was seen on the stem and leaves almost all of these seedlings developed strangulation later, and these seedlings when transplanted showed symptoms of the stunt disease.

TABLE 2. *Symptoms of Disease in Stem and Leaves and Strangulation at Soil Level*

	Strangulated	Not strangulated	Total	Percentages
Plants without disease symptoms	86	33	119	72.2
Plants with disease symptoms	38	4	42	90.5

Remarks: Plants without symptoms mean those which looked healthy above the ground. Samples were taken from Section 5 of Saneto-Saneto's experimental area. (Date of investigation: 25th April)

When the infected plants begin to appear over the unsterilized seedbeds, some seemingly healthy plants when transplanted, develop disease symptoms later. In comparison with those grown on sterilized seedbeds, they are generally not uniform in their growth having smaller stems and narrower leaves. When the temperature is high, the symptoms are hardly discernible, but the growth is retarded and results in late crop. When grafted during high summer temperatures, the symptoms of the disease cannot be seen.

As regards the extent of distribution of this disease, it is known in Kagoshima Prefecture in the Kyushu area. A sample collected by Mr. Masahiro OKUURA in the vicinity of Hirado City, Nagasaki Prefecture and another one collected and sent from the same area by Mr. Seiichi YONEMOTO proved to be infected by this disease. As mentioned above, almost every district offered samples and this fact tends to make us believe that the disease in question exists everywhere in the area. Again, Atsumi Peninsular Area in Aichi Prefecture and almost all over Kanagawa Prefecture this disease can be seen. Samples were also collected in Namekata-gun of Ibaraki Prefecture and the Ohazama Area of Iwate Prefecture.

Discussion

1. The disease having been determined new to the country, all possible methods of research and experiment were incorporated in the plan to determine the manner of development and the ways and means of preclusion and prevention of the disease.

2. Our preparatory research indicated that this disease originates in the seedling stage. In the planning of the research, all possible routes of infection was considered. For such experiment planning, detailed and careful preliminary investigation was considered important.

3. According to Table 1, infection of the plants was very rare in the seedbeds whose soil was sterilized. It is, therefore, considered that the virus of the disease is conveyed with the soil in the seedbeds. Throughout the five experimental areas, a large number of disease cases were seen in Sections 2 and 5, and it was very rare in Sections 1, 3 and 4. It is evident that steam sterilization of seedbed soil can prevent almost completely the development of this disease. Others such as heat materials, seedling cloth and other seedbed materials were not considered possible infetcion sources, nor were insects.

4. It is thus now evident that this disease is conveyed with the soil, but no details regarding this soil infection are known and they are for future study.

5. In the non-sterilized seedbeds soil of Sections 2 and 5, infected plants were seen evenly over the area, while on the sterilized seedbed soil of Sections 1, 3 and 4 they were very few and, if any, they developed in groups. This is perhaps due to incomplete sterilization in parts of the soil, or to the fact that infested soil was mixed after sterilization. When few plants are infected, they are in groups, so by avoiding the seedlings around the infected ones healthy plants were easily pick out at the time of transplanting.

6. Though no infection is seen after transplanting into fields, plants can develop the symptoms. It is, therefore, important to make good selection of healthy seedling at the time of transplanting, and most important to prevent the disease by sterilizing the soil for seedbeds.

7. Slight tendency was seen that bad drainage and consequent high soil moisture had something to do with the development of the disease, but this cannot be considered direct or important factor. The relation of the rate of growth of the seedlings and the nutritional condition to the development of the disease was not defined in this experiment. It can be said, however, that symptoms of the disease appear earlier in the plants that

were well grown and well nourished.

8. The disease starts when the leaves are from 6 to 7, and when they become darker and the internodes stop growing. The base of the plant, near the soil level where stem changes to root, becomes necrotic and strangulated, and when bent, it easily breaks at the point. On the leaves faint chlorosis or necrotic patterns spread sometimes beyond the vein. In the fields, besides the fact that the internodes would not grow, stems are smaller and the leaves are darker and narrower. All these symptoms are seen in all diseased plants, but strangulation at the soil level is most common.

9. On diseased plants, when transplanted, leaves grow as rosette from the shortening of the internodes. When the temperature is high, those slightly infected do not necessarily became rosette, but their leaves are always darker and narrower.

10. The tobacco disease with these symptoms has been known as "Chijimi" (shrink disease) and by other dialectic names but by no formal name based on any study. From this study a name "Tobacco Stunt Disease" is proposed for this disease. The infected plants not only appear shrunk, but stop growing and stand rosette on the ground, and hence our nomination. Disease having comparatively similar symptoms to the disease in question and soil borne are tobacco rattle (QUANJER, 1943; VAN DER WANT, 1951) and tobacco necrosis (PRICE, 1938) of the tobacco plant, and of other plants there are wheat mosaic (MCKINNEY, 1946) and lettuce big-vein (PRYOR, 1946) and others. There are few other soil borne virus diseases but they are different from this disease in question.

11. The distribution of this disease is reported from Kagoshima Prefecture in the south to Iwate Prefecture in the north, i. e., actually throughout the country.

SUMMARY

This report deals with the findings of the research party organized to study the new disease "Tobacco Stunt".

1. To combat the tobacco stunt disease caused by a hitherto unknown causal agent, a well equipped research party was organized. A control method to eradicate the diseases was successfully established.

2. As it is a new disease of tobacco, it was named "Tobacco Stunt Disease".

3. It is a so-called soil borne disease occuring in the seedbed soil. It is possible to control it almost perfectly by sterilizing the soil with steam.

4. Transmission of the disease in the field, or by insects or by other materials of seedbed construction was not apparent.

5. It is supposed that the disease is distributed all over the tobacco districts of Japan, and it seems to be an unknown disease in foreign countries.

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EXPLANATION OF I-PL. I

Fig. 1. Symptoms on a seedling infected with the tobacco stunt disease.

Fig. 2. Development of the diseased plants on sterilized seedbed soil. They are seen in groups as marked with straw.



Fig. 1



Fig. 2

EXPLANATION OF I-PL. II

Fig. 1. Development of the diseased plants on non-sterilized seedbed soil. Those marked with straw are diseased ones.

Fig. 2. Symptoms on seedlings infected with the tobacco stunt disease. The top leaves are twisted, and angles of the leaves against the stem greater than usual.



Fig. 1



Fig. 2

HIDAKA, Z. (1956). *Bull. Hatano Tobacco Expt. Sta. No. 40, 13–17.*

STUDIES ON THE STUNT DISEASE

II. INVESTIGATIONS ON THE DAMAGES OF THE TOBACCO LEAF BY THE TOBACCO STUNT DISEASE

By Zyun HIDAKA

INTRODUCTION

From the large scale experimental studies which was started in 1949 a satisfactory control measure for the tobacco stunt disease was established. This fact was reported in the previous article. There is however no reliable information is available on the damage of this disease. Although it is gradually increasing now up to about 1949 there were only a few cultivators of bright tobacco. The disease in question is often seen on this variety, especially the "Bright Yellow" variety. In 1949 this disease was very pronounced in where. Investigations were made on the damage during 1949 and 1950. As the disease was not generally known in 1949, the cultivators unaware of the disease transplanted infected seedlings. Accordingly the damage seen in 1949 was the result when no control method was applied. Later with the knowledge of the disease the damage decreased. The present report illustrates the effects of the control method on a disease. When conditions are favourable remarkable effects of the methods established could be seen. The results are explained below.

INVESTIGATION ON THE DAMAGE AND ITS ESTIMATION

In 1949 repeated cases of the tobacco stunt disease were seen in Kanagawa Prefecture, especially at Kitahatano-mura, Naka-gun, and Nishihatano-mura, and Kamihatano-mura of Ashigarakami-gun. Without the knowledge of this disease nor of its damage, many cultivators planted infected seedlings. The area under cultivation of bright tobacco (flue-cured tobacco) was rather small at that time, and the total damage was not too serious. But the infected ratio was great and the cultivators of those fields suffered from serious damage. In 1950, they came to know of this disease fairly well and made good selection of seedlings. Especially in Kitahatano-mura, a cultivator whose seedlings were badly infected on the seedbeds took over healthy ones from other cultivators and transplanted them on the field with the results that there were hardly any damage done in the village. In Kamihatano-mura, however, there was considerable damage. In 1951,

all the villages of Kitahatano, Nishihatano and Kamihatano saw this disease, and they introduced from other sources about 100,000 seedlings. Few cultivators transplanted infected seedling, and the loss was very negligible.

A survey of loss suffered by the five villages in the Hatano District is shown in Tables 1 and 2. The information was gathered in person from representatives of cultivators.

TABLE 1. *Damage done by the tobacco stunt disease Hatano District in 1949.*

	Area	¥ per *tan*	*kg* per *tan*	¥ per *kg*
		¥	*kg*	¥
Undamaged fields	284,2,21*	35,911.33	166.1	216.20
	(73.65)	(100.00)	(100.00)	(100.00)
Damaged fields	101,6,25*	22,050.32	105.5	209.01
	(26.35)	(61.40)	(63.52)	(96.67)

* *Tan, Se, Bu*

Remarks: Loss by other reasons included in undamaged fields. Area and amount of money are as recorded on official records kept in Hatano Branch Office of the Japan Monopoly Corporation. ( ) shows the ratio.

Amount of loss (Q) Damaged Ratio (P) Undamaged area (N)
Damaged area (D) Damaged area ratio (E)
Undamaged area yield per *tan* (A) Damaged area yield per *tan* (B)
Loss ratio (S)
Index number of purchase price for leaf tobacco to that of 1949 (L)

$$Q=(A-B)\times D\times L$$

$$Q_{49}=(35{,}911.33-22{,}050.32)\times 101{,}6{,}25\times 1.00=¥1{,}409{,}388$$

$$P=\frac{A-B}{A}\times 100 \quad P_{49}=\frac{13{,}861.01}{35{,}911.33}\times 100=38.60\%$$

$$E=\frac{D}{N+D}\times 100 \qquad E_{49}=26.35\%$$

$$S=\left(\frac{D}{N+D}\times\frac{A-B}{A}\right)\times 100 \qquad S_{49}=10.17\%$$

TABLE 2. *Damage done by the tobacco stunt disease in Hatano District in 1950.*

	Area	¥ per *tan*	*kg* per *tan*	¥ per *kg*
		¥	*kg*	¥
Undamaged fields	647,1,08	38,619,29	203.6	189.68
	(90.57)	(100.00)	(100.00)	(100.00)
Damaged fields	67,3,28	27,475.89	162.9	168.67
	(9.43)	(71.15)	(80.01)	(88.92)

Remark: See Table 1.

$Q_{50}=(38{,}619.29-27{,}475.89)\times 67{,}3{,}28\times 1.00=$ ¥750,987

$$P_{50}=\frac{10{,}693.40}{38{,}619.29}\times 100=28.85\%$$

$$E_{50}=\frac{67{,}328}{714{,}5{,}06}\times 100=9.43\% \qquad S_{50}=2.72\%$$

TABLE 3. *Area in cultivation and yield of bright tobacco in Hatano District*

Year	Cultivation area	¥/*kg*	*kg/tan*	¥/*tan*	Yield index (1949: 100) (L)	Estimated loss (On basis of 1949 loss)
1951	1115, 3, 29*	226.0	207.9	47, 619	109	5, 887, 856
1952	1172, 4, 06	301.6	190.0	57, 342	104	7, 110, 558
1953	1518, 5, 03	306.8	152.5	46, 744	100	7, 218, 748
1954	1916, 1, 00	296.6	175.4	51, 941	100	10, 121, 606
1955	2551, 8, 12	290.2	236.6	68, 594	100	17, 801, 661

* *Tan, Se, Bu*

Remarks; Yield ratio of tobacco leaves versus pervious year is:

Year	1950	1951	1952	1953	1954	1955
Increase	12.0	19.6	5.0	3.7	0.0	0.0

DISCUSSION

The percentage of damaged area in Hatano District was 26.35 percent in 1949 and decreased to 9.43 percent in 1950. Loss ratio, decreased too, from 10.17 percent to 2. 72 percent. This is perhaps due to the knowledge of the disease given by our ivnestigation of 1949. But if it were not for this investigation, same degree of damage would have continued yearly. In fact, in 1950, 1/3 of the total seedbed area was infected in Kitahatano-mura and the majority of these infected seedlings were not transplanted. On those that were planted, they were replaced later by healthy ones grown by other cultivators. If they did not know of the stunt disease as was before 1949, the same damage must have been repeated. Even in 1950, those who did not apply the control methods for this disease suffered some 28.85 percent decrease in revenue per *tan*. The amount of loss (Q'_{50}) can be calculated, according to Table 2, together with the increase in cultivated area, in the following way.

$Q'_{50}=$ ¥3,143,096

In other words, where there would have been a loss amounting to ¥3,143,096, the loss was only ¥750,987. The difference of ¥2,393,109 can be considered the sum gained by controlling the disease. There is no difference

in the amount of revenue between the years 1950 and 1954, but the quality standard was raised, and thus it means that there was a 12 percent increase actually. In 1949 infected leaves were only 3.33 percent lower in price than healthy leaves per kilogramme, but in 1950 it was lower by 11.08 percent. This tendency was more remarkable in 1951 and subsequent years, and the price difference per kilogramme must be even greater. In 1949 decrease per *tan* was 36.48 percent, but since the figure per kilogramme was not too low, the loss was only 38.60 percent. If the disease had not been under control, the loss must have been more serious after 1951, and it would have been perhaps impossible to increase the acreage and to prosper in this area as we have today. Since 1951 only a few diseased seedlings in few seedbeds is observed in the Hatano District every year. These diseased plants are seldom transplanted, and actually it can be presumed that the loss is negligible. If we calculate the loss in 1949 from the results of 1951 and 1955 as shown on Table 3, we get the following. In 1951 there were some cases of the stunt disease in some seedbeds in Kamihatano-mura and Nishihatano-mura, but the cultivators took over healthy seedlings from other sources, so that the above-mentioned five villages were almost completely free from loss. According to Table 3, in 1951 the cultivation area of bright tobacco was 111 *cho* 5 *tan* 4 *se*, with the revenue of ¥47,619 per *tan*. Now if we had the same degree of damage as we did in 1949, the loss (Q'_{51}) would have been: thus,

$$Q'_{51}=111,5,4\times47,619\times0.1017\times1.09=¥5,887,856 \quad \text{(Loss amount)}$$

Further in 1955 the amount of loss (Q'_{55}) would have been, on the basis that the cultivation area was 255 *cho* 1 *tan* 8 *se* 12 *bu* and the revenue being ¥68,594, and on the assumption that the same degree of damage was done to the crop: thus,

$$Q'_{55}=255,1,8,4\times68,594\times0.1017\times1.00=¥17,801,611 \quad \text{(Loss amount)}$$

This means that even only in the Hatano District alone the damage would have been tremendous. The total amount of estimated loss of 1951 through 1955 is ¥48,140,429. If it were not controlled, it causes damages not only for one year but year by year. Besides this could have happened in all bright tobacco cultivation areas throughout the country, i.e., Seto Inland Sea Coast Area, Kyushu, and Tokai Districts. Today there is no damage reported from this disease. What was gained from the control measures, therefore, was not small as described above. ¥800,000 was expended for the research and investigation activities as explained in Report I. This was amply recovered in 1950 by the yield received from 71 *cho* field of the

five villages of the Hatano District alone. The bright tobacco has been increasingly cultivated so that the area now covers more than one half of the total tobacco fields. The result of the research has thus brought about immeasurable profit.

SUMMARY

In Hatano District, Kanagawa Prefecture, Japan, the damage caused by the tobacco stunt disease in 1949 was estimated to have reduced the output by about 10.17 percent in price. As a result of the control method described in previous report, this reduction in output decreased to 2.72 percent in 1950 and to about zero percent in 1951. This means a gain of 2.39 million yen in 1950. As the Bright Yellow tobacco area continues to increase year after year, the damages calculated at the rate of 1949 would be enormous. By the cooperative effort of all concerned in effecting the control method, there has been no damage in 1951, and the resulting gains in output calculated on the 1949 basis amounted to 5.88 million yen for 1951 and 17.80 million yen for 1955.

HIDAKA, Z. (1956). *Bull. Hatano Tobacco Expt. Sta. 40, 19–23.*

STUDIES ON THE TOBACCO STUNT DISEASE

III. ON SYMPTOMS AND CAUSAL AGENT OF THE TOBACCO STUNT DISEASE

By Zyun HIDAKA

A brief account of the symptoms of this disease was given in Report I. The present report further describes the symptoms in detail. We are also going to report on the results of the inoculation experiment and the research on the pathogen.

SYMPTOMS

About 40 days after seeds are sown, when the plant has 6 to 7 leaves, the first symptoms are seen. They develop most conspicuously about 4 to 5 days before the transplanting time. The first indications of the disease in the seedbeds are: folded top leaves a little twisted, and expand wider than normal in angle of the leaves to the stem (see III-PL. II, Fig. 1). By this time veins become veinclearing, and the veinlets of expanded leaves (usually the biggest leaves) become veinclearing. This state develops netwise from the base to the tip of the leaves changing the infected part into pale yellow and in a few days into brown necrosis (see III-PL. I, Figs. 1 and 2). On the surface of the infected leaf are seen necrotic spots scattered around, but on the back of the leaf veinlets are collasping with necrosis all over the leaf like a net (see III-PL. II, Figs. 1 and 2). The necortic sports on the leaf are each a small irregular square, but they usually range in a shape of a ring. Later, these spots dry up and change to white, but the parts encircled with these necrotic spots change to pale green or yellowish green (see III-PL. II, Fig. 2 and III-PL. III, Fig. 2). Necrosis is not only seen on the leaves, but also on the petioles, mid-ribs and veinlets, and the necrosis on the leaves sometimes spreads beyond veinlets (see III-PL. III, Fig. 1). Internally, vascular bundles are not free from necrosis, and sometimes the part of the plant above the necrotic mid-rib or petiole withers and dies. The part of the stem at soil level, too, usually suffers from necrosis in its cortex surrounding the woody part, the infected part changing to brown. When sections from this infected part are taken and examined under a microscope, we can clearly recognize necrotic rings stretching on vascular bundles of the stem. This symptom is most conspicuous around the soil

level part, and it is here that the symptom first appears, showing a necrotic circle particular to this disease (see III-PL. IV, Fig. 1). Tobacco leaves showing these symptoms are of darker colour and often show mottling and are easy to break (see III-PL. III, Fig. 2). The symptoms are most remarkable on the leaves in the front and the back of the largest leaf and gradually spread to upper leaves. They seldom spreads down to the lower leaves.

Towards the end of seedbed period and in the first part of the growth period after having been transplanted in the field, net-like necrosis on the veinlets causes to develop, and the top leaves gradually start growing. Due to the ill-growth of the internodes, however, the whole plant is stunted and looks like a rosette (see III-PL. V, Fig. 1). On these leaves, seemingly so far, first appear faint spots and then necrosis in waves appears parallel to the mid-rib. This necrosis changes from brown to white (see III-PL. V, Fig. 2 and III-PL. VI, Fig. 2). The surface of the leaves becomes uneven. The leaves shrink and roll inward or show mottling (see III-PL. VI, Fig. 1).

After the final soil dressing in the field, together with the rise in the temperature, the symptoms become less conspicuous, and even the newly grown leaves do not show much necrosis. The symptoms of this disease, regardless what stage the plant is in, have much to do with the temperature. Experiment showed that an infected plant, when kept for several days at the temperature of 25°C, grows seemingly healthy, and the part which growth later do not present any indication of the disease. Still, however, the leaves of the infected plant in the field which masked the symptoms are of rather darker colour and the internodes shorter with narrower leaves. Some remain dwarfed stunted and are slow in blooming, though they finally do bloom and bear fruits. Even when the indications are slight, growing apparently healthy, if kept until October, the plants begin showing typical symptoms of this disease.

X-body and other intracellular inclusions have not so far been recognized.

Inoculation experiment

The writer earlier (1951) stated that the virus for this disease could perhaps be inoculated by sap. This, however, is believed to have been due to the fact that the virus for this disease was mixed with some other virus, and the writer has to amend his statement. We have carried out, so far, sap inoculation and other methods of inoculation for the virus diseases, i. e., (1) sap inoculation with carborandum, (2) Koba's glass micro-injector method, (3) perforation method using an infected leaf and a healthy leaf one upon another, (4) inoculation using a dodder, as well as insect vector

experiment by aphids and *Gallobelicus crosieornis* DIST. None has succeeded to the present. As reported in Report I, our study in the relation of insects and this disease revealed nothing considered relevant to this disease. Besides the soil borne, the only artificial transmission of this disease possible is grafting. Grafting succeeds 100 percent either with a healthy scion on an infected stock or an infected scion on a healthy stock. The host range is limited to tobacco, as far as known today, and will be explained in Report VII. Of all varieties of tobacco, "Bright Yellow" variety is most easily infected. The degree differs much according to the variety. Seed transmission was not recognized in our two experiments.

PATHOGEN

First we grew tobacco plants on infested soil and some on sterilized soil, and when those on infested soil indicated symptoms of the tobacco

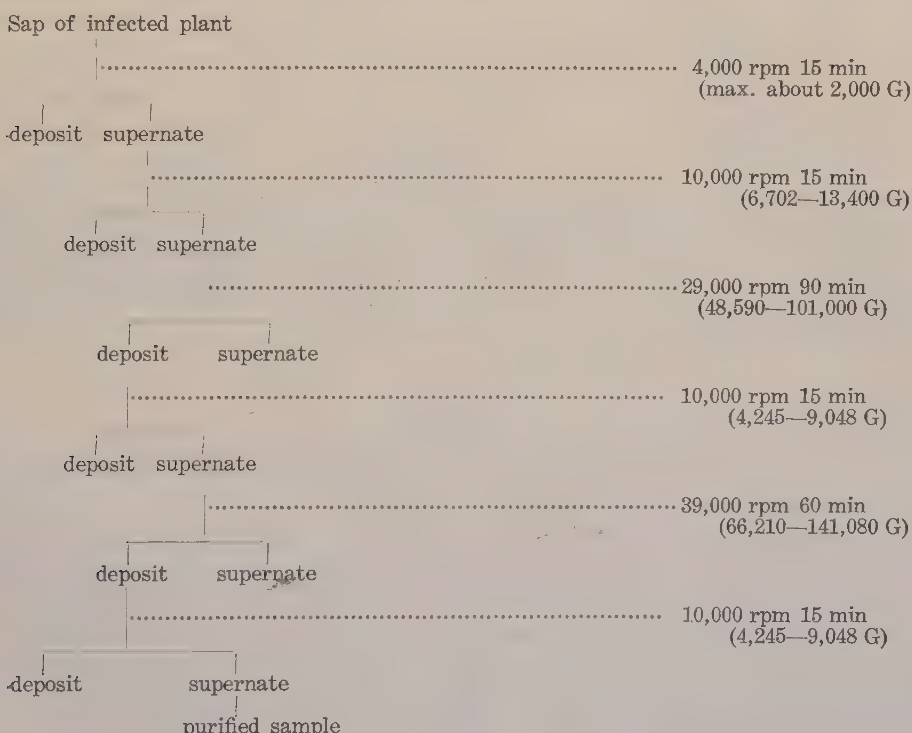


Figure 1. *Procedure of differential centrifugal purification for the tobacco stunt virus*

stunt disease and when the healthy plants were ready for transplanting, we cut the infected plants at the soil level and did the same thing with healthy plants on the sterilized soil (they were all healthy). These we froze overnight in an ice-box at the temperature of −15°C. Then we macerated them in a meat-grinder. The sap thus acquired was filtered through double gauze and then purified by centrifugal method as illustrated above.

By this method we got fairly thick material from sap of the infected plants, as in the case of tobacco mosaic virus which is of brownish protein colour. From the sap of healthy plants in the process second from the last between 66,210 and 141,080 G, there was hardly any deposit. So far, the same experiment was repeated 28 times and always had the same result. A part of the purified material, thus acquired, was mostly examined under an electron microscope. The results were same as shown in III-PL. VIII, Figs. 1 and 2. Uniform particles which were absent in the case of healthy plants were seen. The size of these particles was considered about 18 mμ and spherical in shape. The pictures in III-PL. VIII, Figs. 1 and 2 seem quite different from each other, but this is due to the difference in kinds and amount of metal used in shadow-casting in preparing the preparation. This has been reported separately (HIDAKA, 1955). These particles are considered to be the virus of this disease. Since sap inoculation is impossible, it is also impossible to inoculate this purified material. Speaking strikely, it cannot, therefore, be confirmed that this is the virus. But the results of the inoculation and guessing from the disease symptoms, it may safely be said that this is one of the virus diseases. The fact that we always acquired the same result from 28 experiments leads us to believe that what we recognized under the electron microscope must be particles of the virus for this disease.

SUMMARY

The writer studied the symptoms, inoculation method, and pathogen of the tobacco stunt disease in the present paper.

1. When the plants are about 40 days old, the symptoms begin to develop at first as a clearing of the veinlets followed by necrosis. It often shows a ring like pattern linked with each other. It shows crinkles on young leaves, deformation of leaves, downward rolling, and mottling. It develops a specific necrotic banding in the stem at ground level, and ring like brownish necrosis in the vascular bundle. It takes on the shape of rosette due to the ill-growth of internodes.
2. The disease is inoculable artificially by grafting only.
3. From the sap of a diseased plant, the writer obtained a purified sedi-

ment of protein colour by the ultracentrifuge fractionation. The material as observed under the electron microscope consists of uniform spherical particles, about 18 mμ in diameter.

4. From the results of above experiments, the disease is considered to be caused by a new virus.

References

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EXPLANATION OF III-PL. I

Fig. 1. The leaves showing the clearing of the vein and partial necrosis of the veinlets. The plant is "Bright Yellow" variety. (All plants shown below are of the same variety.)

Fig. 2. The veinlets are necrotic, first in small spots and later these spots connect themselves in rings. The part encircled by the rings changes to pale green or yellowish green.



Fig. 1

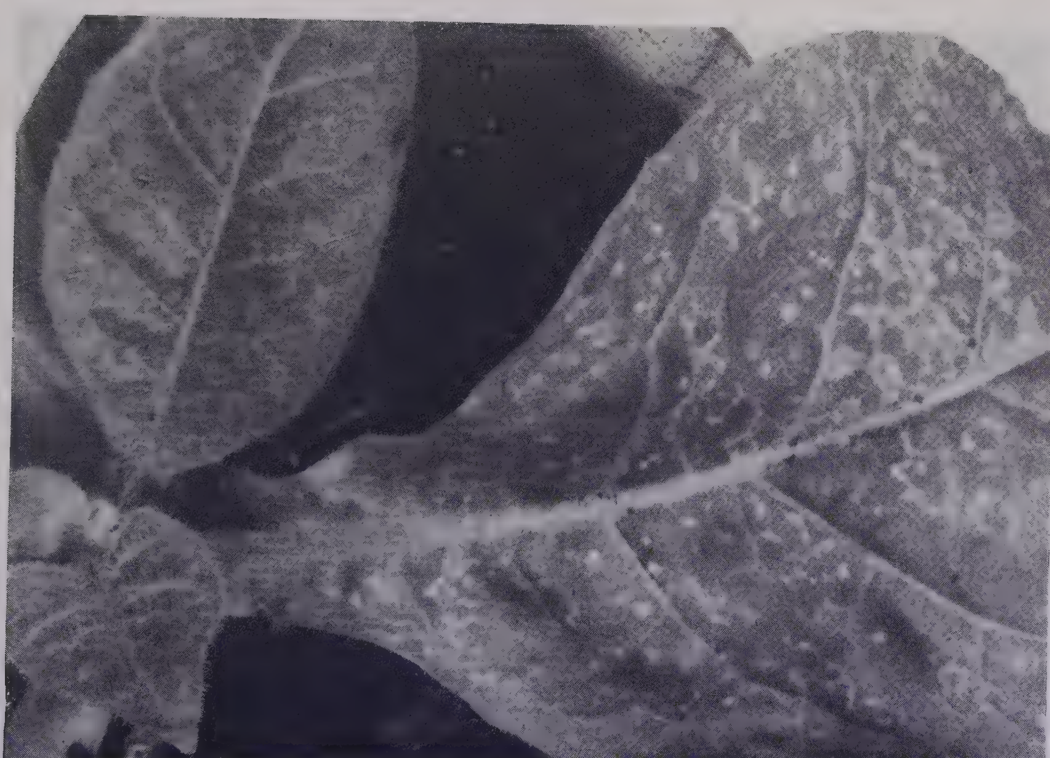


Fig. 2

EXPLANATION OF III-PL. II

Fig. 1. Top leaves are twisted and changed to yellow, and they have a larger angle than normal to the stem. Necrosis in veinlets starts from the base and develops upward. Sometimes the tip of the leaves remains free from the symptoms or necrotic spots in rings appear here.

Fig. 2. Top leaves have a larger angle than normal to the stem, and the necrotic spots in rings are seen.



Fig. 1



Fig. 2

EXPLANATION OF III-PL. III

Fig. 1. Necrosis on the leaves mainly parallel to the mid-rib spreads like waves over vein.

Fig. 2. Due to necrosis in the mid-rib and vein, the leaves become uneven. Top leaves, with their mid-rib developed necrosis, curl underside from the point, and are hampered in their growth.



Fig. 1



Fig. 2

EXPLANATION OF III-PL. IV

Fig. 1. Necrosis at the soil level. The one not marked is the healthy plant.

Fig. 2. The comparison in growth of a diseased plant and a healthy one.

Left: Seed sown on the infested soil.

Right: Seed sown on the sterilized soil.

Both are shown about 70 days after the seed were sown.

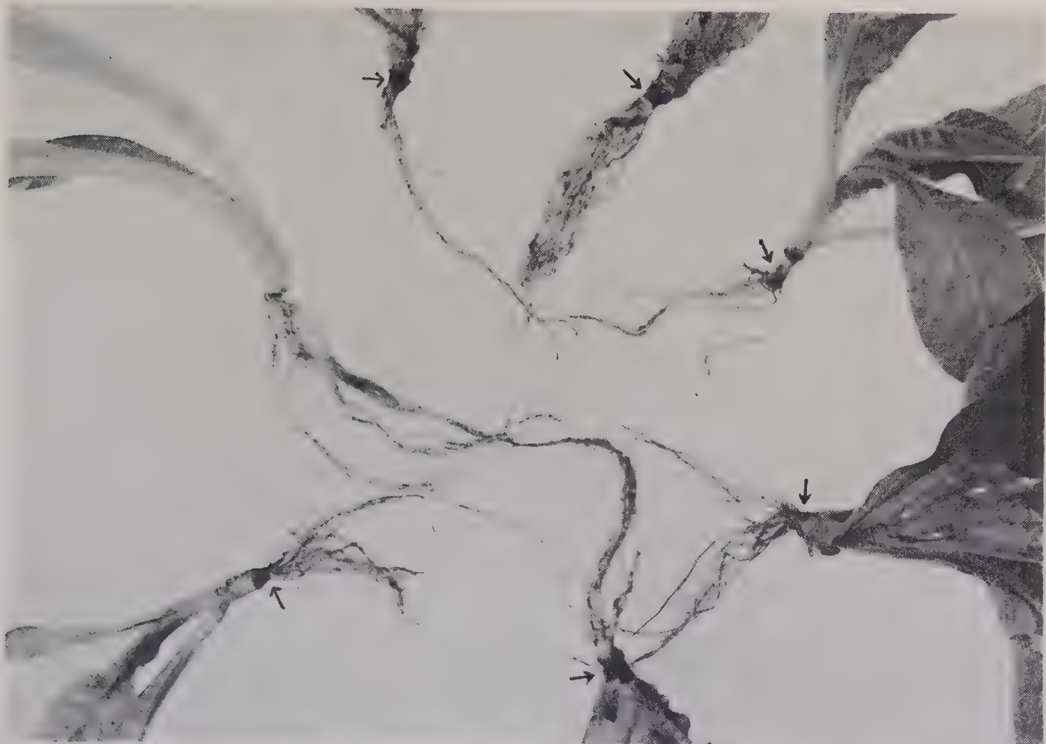


Fig. 1



Fig. 2

EXPLANATION OF III-PL. V

Fig. 1. A diseased plant with its top leaves fairly grown. Lower leaves show their early symptoms.

Fig. 2. Necrotic pattern appears mainly parallel to the mid-rib and runs over the vein. Some show ring-like necrosis at the tip of the leaf, though the top leaves look free from symptoms as the temperature rises.



Fig. 1



Fig. 2

EXPLANATION OF III-PL. VI

Fig. 1. Top leaves are fairly grown, but the leaves are conspicuously uneven, shrunk, discoloured partly and are stunted.

Fig. 2. Necrotic spots shaped like waves.



Fig. 1



Fig. 2

EXPLANATION OF III-PL. VII

Fig. 1. Symptoms in the fields.

Fig. 2. Symptoms in the fields. Some are stunted and those which are not stunted are ill-grown and lean.



Fig. 1



Fig. 2

EXPLANATION OF III-PL. VIII

Fig. 1. The tobacco stunt virus under an electron microscope. 1: 58,800. Thick shadow-casting with chromium.

Fig. 2. The tobacco stunt virus under an electron microscope. 1: 47,000. Thin shadow-casting with chromium.

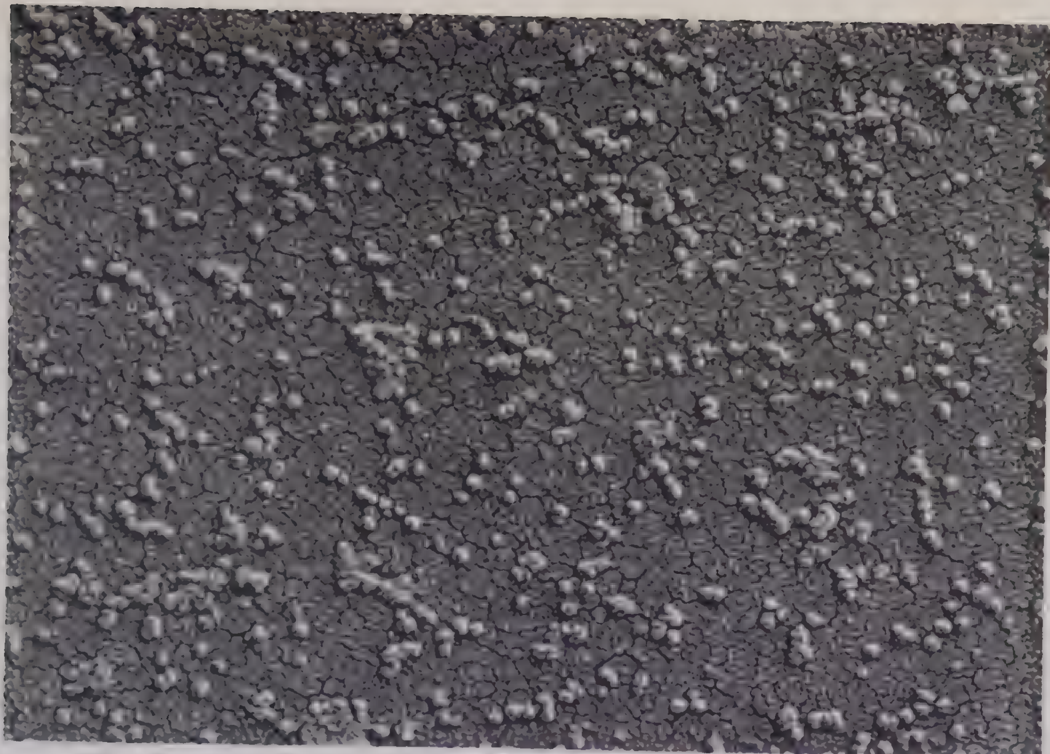


Fig. 1

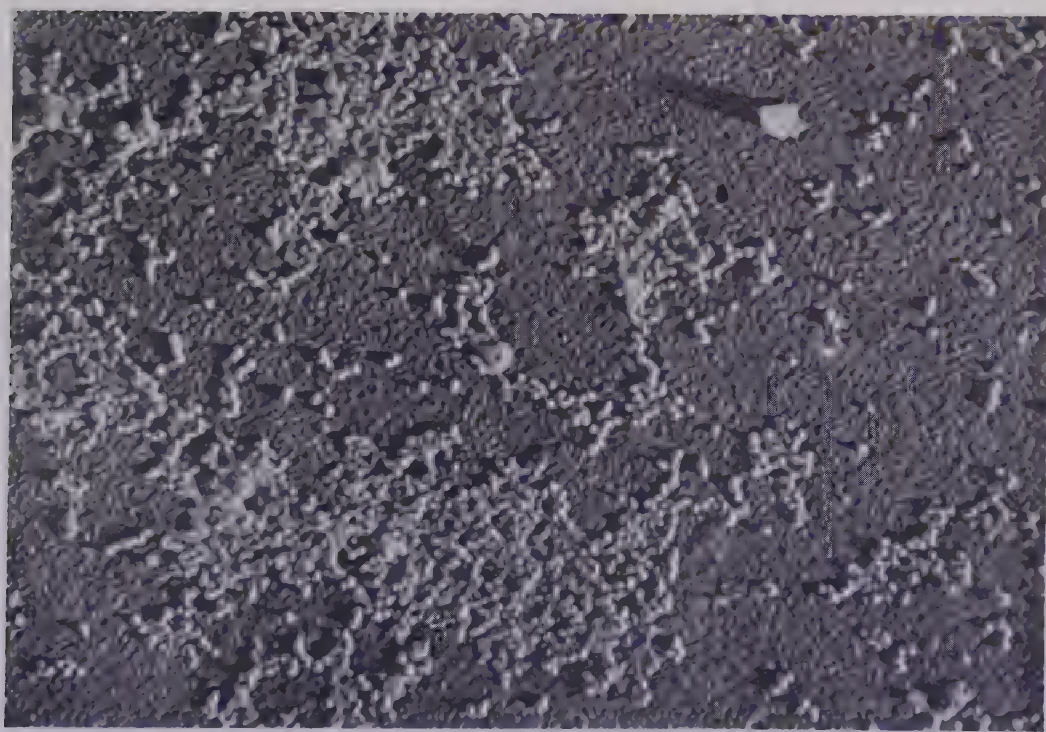


Fig. 2

HIDAKA, Z. & UOZUMI, T. (1956). *Bull. Hatano Tobacco Expt. Sta. 40, 25–30.*

STUDIES ON THE TOBACCO STUNT DISEASE

IV. ON WHEREABOUTS OF THE TOBACCO STUNT PATHOGENE BORNE BY THE SOIL

By Zyun HIDAKA and Tetsuo UOZUMI

INTRODUCTION

The viruses of wheat mosaic, wheat yellow mosaic, lettuce big-vein, tobacco necrosis, tobacco rattle, tobacco stunt disease and broad bean necrotic mosaic, etc. are known to be transmitted by the soil. The tobacco stunt disease begins to occur in the seedbed soil when the tobacco plants are about 40 days old and have 6 to 7 leaves including the cotyledons. Sometimes the infection is 100 percent, resulting in serious loss to the tobacco production. In this experiment the problem of carrying-over of virus in the seedbed into the following year is taken up, and we carried out a few experiments by the way of determining this.

EXPERIMENTAL

1. On the presence of tobacco stunt virus in the soil

From the seedbed on which the disease developed, samples of (A) seedbed surface soil, (B) seedbed sub-soil, (C) heat materials (see Figure 1), (D) garden soil on which the seedbed was constructed upon, (E) field soil around the diseased plant, and (F) field soil free from the disease were taken. Sterilized farmyard manure was mixed with (A), (B), (D), and (E) in the ratio of 1:1. Sterilized soil was mixed with (C) in the same ratio. Soil samples thus prepared were placed in sterilized 10 in. pots, and were planted with tobacco seeds (Bright Yellow variety) to observe the development of the disease.

As shown on Table 1, all treatments except the one sown on healthy soil showed the development of the disease, i.e., from surface soil, sub-soil, heat materials, garden soil and the field soil around the diseased plant. The percentage was especially great on the surface soil and heat materials.

2. Infection of the tobacco stunt disease via sub-soil and heat materials

In the experiment of autumn 1954, seedbeds were prepared by using the surface soil on which the disease had appeared the previous spring. This

top layer was spread over three types of soils, namely, loam, clay soil and river sand. The result of this experiment is given in Table 2.

Samples of above soils and fallen leaves material were taken and stored in earthen pipes.

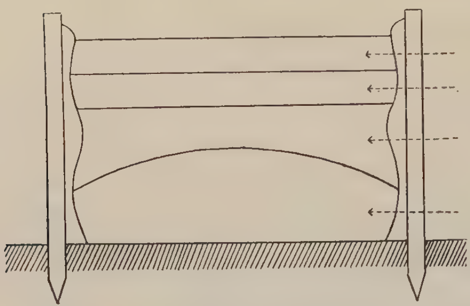


Figure 1. A sketch of a section of a seedbed

TABLE 1. *Seedbed materials, garden soil and field soil on the development of the tobacco stunt disease*

Materials used	Sep. to Nov. 1952			Sep. to Nov. 1953		
	No. of plants	No. of diseased plants	Percentage diseased	No. of plants	No. of diseased plants	Percentage diseased
A. Surface soil	54	14	26.0	110	59	53.6
B. Sub-soil	—	—	—	147	3	2.0
C. Heat materials	138	53	38.4	92	17	18.5
D. Garden soil	169	12	7.1	143	0	0.0
E. Soil around infected plants	202	6	3.0	122	2	1.6
F. Healthy soil	253	0	0.0	—	—	—

Remarks: Above is the result gained from three 10 in. pots examined under indicated conditions. Bright Yellow variety was used for this experiment as being most susceptible to this disease, as in the following experiments.

TABLE 2. *Kinds of sub-soil for the seedbed and the tobacco stunt disease*

	No. of plants	No. of diseased plants	Percentage diseased
Loam*	308	109	35.4
Clay soil*	316	62	19.6
River sand**	290	63	21.7

Remarks: * The layer was 3.6 in. thick.
** The layer was 1.8 in. thick.

We further experimented on the sub-soil and the heat material (fallen leaves).

A. *When the same sub-soil used the previous year was used.*

In spring of 1955 the same sub-soil used the previous experiment descrbide above was used without sterilizing it. The surface which went over the sub-soil had been sterilized with steam. Another seedbed with sterilized sub-soil was prepared at the same time.

B. *When the surface soil was prepared with the previous year's sub-soil*

The surface soil was prepared from the three different kinds of sub-soil, loam, clay soil, and river sand, each of which was mixed with equal amount of steam-sterilized farmyard manure. Steam-sterilized soil was used for the sub-soil in the thickness of 1.5 in.

C. *When the surface soil was prepared from the previous year's heat material.*

The farmyard manure was prepared from the heat material (fallen leaves) used in three different kinds of seedbeds, i.e., of loam, clay soil and river sand. When the manure was well fermented, it was siftd through a 1.3 cm mesh sieve, and mixed the small clods with the same amount of steam-sterilized manure. 1.5 in. thickness of sterilized soil was used for the sub-soil to determine the relation of development of the disease and the heat material. The results obtained from the experiments A, B and C are as shown in Table 3.

Dividing the sub-soil of the seed-bed where the disease appeared in the previous year into two parts, the first half was retained and reused as sub-soil (Case A.) ; and on the second half it was mixed with the same amount of sterilized farmyard manure (Case B.). In both of these cases the percentage of development of the disease was small. In Case B, compared with A, though the sub-soil was diluted by mixing with the same amount of sterilized farmyard manure, the disease development percentage was great. This prove the contamination of the sub-soil from the previous year's infected surface soil of the seedbed, which had much to do with the development of the disease; and on the contrary, even if the contamination is great the percentage of infection is small if the contaminated soil was put at some distance from the surface of the seedbed. The fallen leaves used as the heat material of the seedbed in the previous year was also contaminated, and if this was used in the surface soil the infection percentage is high. We saw less infection on the surface soil made from the loam seed-

bed sub-soil and fallen leaves. This was perhaps due to the fact that we made the sub-soil in the previous year 3 in. thick, which was twice as thick as in other kinds of seedbed.

TABLE 3. *Development of the disease by sub-soil and heat material of seedbed where the disease was seen in the previous year*

(Feb. to May, 1955)

	Seedbed materials	No. of plants	No. of diseased plant	Percentage diseased
A. Sub-soil	Loam	214	2	0.9
	Clay soil	218	3	1.4
	River sand	204	4	1.9
	Sterilized soil	222	0	0.0
B. Sub-soil	Loam and sterilized surface soil	185	32	17.3
	Clay soil and sterilized surface soil	183	78	42.6
	River sand and sterilized surface soil	179	104	58.1
C. Fallen leaves	From: loam bed and sterilized surface soil	200	13	6.5
	From: clay bed and sterilized surface soil	200	46	23.0
	From: river sand seed-bed and sterilized surface soil	225	37	16.4
Sterilized surface soil	Sterilized surface soil	190	0	0.0
Previous year's surface soil	Infected soil	222	183	82.4

DISCUSSION

As was explained by these experiments, the tobacco stunt disease is one of the soil-borne virus diseases on the seedbeds. When the surface soil, sub-soil and heat materials used in the seedbeds were all contaminated, they show a high infection percentage, and become a possible source of future cases of the tobacco stunt disease. In practice, however, the old surface soil or sub-soil is never used again in new seedbeds. As a material for next year's surface soil the cultivators generally use the heat material of the seedbeds in making farmyard manure. It is considered, therefore, that this is the source of next year's infection. The garden soil, too, is not free from contamination. It is very possible that this is mixed in preparation of the surface soil for next year. We have to take especial care not to mix unsterilized garden soil into the surface soil. Report IV deals with the

fact that the disease was seen on the infested soil diluted 127 times with sterilized soil. This shows that great care should be taken, in order to prevent infection, not only to sterilize the suface soil to be used but also in carrying the sterilized surface soil to the seedbeds. It was not recognized that the virus increases in the field soil. It is also not practically possible that the contaminated field soil is mixed in the surface soil there. This degree of contamination in the field may not be a powerful disease source. The virus is perhaps carried from the seedbed to the field in the soil attached to the root of the seedling when transplanted, and is not considered to increased later. In this degree we believe it will not be transmitted in the field. IKATA and KAWAI (1940) in their study of wheat yellow mosaic, and VAN DER WANT (1951) in his study of tobacco rattle, suggested a possibility that the virus may exist adsorbed in soil particles. The writer's experiments, however, do not necessarily verify this suggestion, for, if it were so, it should be stronger in sub-soil rather than in fallen leaves and moreover, in clay soil rather than in river sand among the sub-soil. The trends of the virus of this disease as described in Report III, and also the results of chemical treatment explained in Report IX tend to deny that the virus itself is adsorbed in the soil particles.

SUMMARY

In this report, experiments with seedbed material, garden soil and field soil were conducted to ascertain the whereabout of causal agent of the tobacco stunt disease.

1. In the seedbed where the disease had developed, the surface soil, sub-soil, and fallen leaves used as heat materials were all found infested. Of these materials, as the fallen leaves used as heating materials are used against the subsequent year as an ingredient in the preparation of surface soil, they are considered to harbour the causal agent. At the same time, there is also a possibility for the garden soil to transmit the disease, and it is necessary to control the soil by disinfecting it and by taking care not to mix it.

2. The disease developed only when the infested soil was in surface of the seedbed.

3. When loam, clay soil and river sand were used as materials for seedbed, and infested with diseased surface soil, the materials did not show any apparent difference in the infectiveness.

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STUDIES ON THE TOBACCO STUNT DISEASE

V. EFFECTS OF STORAGE PERIODS, AIR-DRYING AND HOT-WATER TREATMENT OF THE INFESTED SOIL ON THE INFECTIVENESS OF THE TOBACCO STUNT DISEASE

By Zyun HIDAKA, Tetsuo UOZUMI and Tadao SHIMIZU

INTRODUCTION

The characteristic of soil borne virus disease, as is known today, is that the virus survives long in the soil and can be the source of infection. In the study of wheat yellow mosaic (IKATA and KAWAI, 1940) the soil remained infectious under indoor and outdoor conditions: three years under natural circumstances of outdoors and four years in dry condition of indoors. With lettuce big-vein (PRYOR, 1946) the virus remained infectious for at least eight years when in air-dried soil. It is important, for the control of this disease, to determine how long the virus for the tobacco stunt disease remains infectious in the soil, and how it is affected by air-drying and hot-water treatments. The following research has been done in this direction.

EXPERIMENTAL

1. Storage period of the infested soil and development of the tobacco stunt disease

In the spring of 1951 at Yokono, Kitahatano-mura, Kanagawa Prefecture, some of the surface soils of the seedbed supporting diseased plants were collected for treatment and observation. Two treatment were made:

TABLE 1. *The disease development from the infested soil stored indoors and outdoors*

No. of years	Experiment dates	Where kept	No. of plants	No. of diseased plants	Percentage diseased
1	Feb.-May, 1952	Out	122	50	40.9
2	〃 1953	In	119	36	30.3
		Out	94	38	40.4
3	〃 1954	In	128	28	21.9
		Out	111	30	27.0
4	〃 1955	In	221	143	64.7
		Out	180	143	23.9

Remarks: Each examination was based on three 10 in. pots. Plants used were Bright Yellow variety throughout this and other experiments.

One was left outdoors under natural conditions and the other was kept dry in a glasshouse. These soils were kept separately from becoming mixed with other soils. In February of each year, a part of the soil from each lot was taken and placed sterilized 10 in. pots and seeds were sown. The results recorded in May of each year as shown below in Table 1.

The soils under both conditions retained infectivity till the fourth year. In the fourth year, the soil kept indoors in dry condition showed more infection, than the one kept outdoors in natural conditions.

2. *Relation of air-drying of the infested soil to development of the tobacco stunt disease*

Experiment I.

In the spring of 1951 samples of surface soil of the seedbed showing many tobacco stunt disease were kept in a earthen pipe used as a container, and were covered from rain. The soil was spread thin over a galvanized iron plate for drying in the sunshine, and was gathered at night. The material was dried for three different periods, i.e., 4 days, 7 days and 15 days. After drying, the soil was put in 10 in. pots and the tobacco seeds were sown.

TABLE 2. *Relation of air-drying on development of the tobacco stunt disease*

(Sep. to Nov. 1951)

No. of drying days	No. of plants	No. of diseased plants	Percentage diseased
4	248	217	87.5
7	221	132	59.7
15	213	159	74.6

Remark: Above is the result obtained from the plants in each of the five 10 in. pots.

Experiment II.

The method used was the same as in Experiment I, but the material used was the surface soil, stored in the same way as in Experiment I, which had been taken from the seedbed where we had many cases of the disease in spring of 1952. The material for A was from this experiment station and that for B was from Mikurube, Kamihatano-mura, Ashigarakami-gun, Kanagawa prefecture, and both were stored here. The experiments were carried out in September, 1952.

TABLE 3. *Relation of air-drying of the infested soil to development of the tobacco stunt disease*

	No. of drying days	No. of plants	No. of diseased plants	Percentage diseased
	4	192	97	50.5
A	7	164	72	43.9
	15	170	76	44.7
	4	180	47	26.1
B	7	203	51	25.1
	15	319	103	32.3
	Control	209	52	24.9

Remark: The results shown above are the total results gained from each of the five 10 in. pots. In the control the stored infested soil was used direct.

Both in Experiments I and II, the sunshine was so strong that the galvanized iron plate was hot, allowing minimum moisture for this method of drying. This, however, had nothing to do with infectivity (See Plate I.).

3. *Relation of the hot water treatment of the infested soil to development of the tobacco stunt disease*

The infested soil was put in a metal beaker (Experiments A and B) and in hemp cloth (Experiment C) with twice the amount of hot water, and then were kept for 20 minutes in a tank of water at an established degree of temperature. The soil was kept stirred during the time. After the treatment, it was immediately put in cold water to be cooled, and then into sterilized pots. Seeds were sown on the soil thus treated.

TABLE 4. *Relation of the hot water treatment of the infested soil to development of the tobacco stunt disease*

	Dates	Water temperature in tanks	Soil temperature	No. of plants	No. of diseased plants	Percentage diseased
A	Feb.-May, 1953	73°C	65–71°C	126	0	0.0
		64	55–60	122	0	0.0
		54	45–50	88	43	48.9
		No treatment		111	39	35.1
B	Sept.-Nov., 1953	65°C	55–61°C	95	14	14.7
		55	47–52	134	94	70.1
		45	40–42	123	74	60.2
		No treatment		123	72	58.5
C	Feb.-May, 1954	70°C	64–66°C	175	0	0.0
		60	55–58	133	0	0.0
		50	44–48	183	3	1.9
		No treatment		193	12	6.2

Remark: Above results are the total number of plants observed from three 10 in. pots.

In Experiment A, materials treated at the temperatures of 45° to 50°C showed no difference in the results from that of non-treated soil, but no infection was seen on the soil treated at 55° to 60°C and 65° to 71°C. In Experiment B, treated materials in the temperatures of 40° to 42°C, and of 47° to 52°C showed no difference in the results from that of non-treated soil, but at 55° to 61°C the infection was less than the non-treated soil. In Experiment C, also, no infection was recognized at the treatments of 55° to 58°C and 64° to 66°C. These facts shown that the disease in question will not develop when the infested soil is treated with hot water of approximately 55° to 60°C for 20 minutes.

Discussion

As in the cases of other soil borne virus disease, in the case of the tobacco stunt disease, too, it is considered that the soil is infested, it will remain infectious for at least four years without decreasing the infectivity. There are many points yet to be determined on the behaviour of plant virus in the soil. Even tobacco mosaic virus, which is considered most stable both physically and chemically does not seen possible to exist in the soil for period years (Hoggan and Johnson, 1936; Hidaka, 1947). In the case of the tobacco stunt disease, no effect was seen from drying the soil for as many as 15 days in direct sunshine. It can be prevented, it is believed, by soil treatment in water of 55° to 60°C for 20 minutes. The fact that the virus for stunt disease, which is considered less stable *in vitro* than tobacco mosaic virus, can remain infective in soil for at least four years tends to make us suppose that this virus survives in the soil in stable condition by some mechanisms different from that of other virus mechanically transmitted.

Summary

This paper reports on the effects of storage periods, air-drying and hot water treatment of the infested soil on the persistence in the infectiveness of the tobacco stunt disease.

1. The tobacco stunt virus was found in persist without showing any decrease in infectiveness for at least four years both in the soil stored in a room and stored out of doors.

2. There was no effect of the sun drying for 15 days on the infectivity of the infested soil.

3. The hot water treatment of the infested soil at 55° to 60°C for 20

minutes was found to be effective enough to check the development of the disease.

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EXPLANATION OF V-PL. I

Fig. 1. Air-drying of the infested soil and development of the tobacco stunt disease.

Left:	dried 4 days
Center:	dried 7 days
Right:	dried 15 days



Fig. 1

HIDAKA, Z. & UOZUMI, T. (1956). *Bull. Hatano Tobacco Expt. Sta. 40, 37–46.*

STUDIES ON THE TOBACCO STUNT DISEASE

VI. RELATIONS OF DILUTION WITH STERILIZED SOIL, POSITION AND DISTANCE FROM THE SURFACE OF INFESTED SOIL IN THE SEEDBED TO DEVELOPMENT OF THE TOBACCO STUNT DISEASE

By Zyun HIDAKA and Tetsuo UOZUMI

INTRODUCTION

It was explained in Report I that the tobacco stunt disease is one of the virus diseases borne by the seedbed soil, and that the seedbed soil is its infection source, but scarcely anything is known on the process of development of the disease. A close interrelation between the position of the infested soil and the development of the disease for such soil borne virus diseases as wheat mosaic and wheat yellow mosaic have already been reported by WEBB (1928), and IKATA and KAWAI (1940). They explained that even when the infested soil was diluted 31 times with sterilized soil the development of the disease was still seen. PRYOR (1946) also reported in his study of lettuce big-vein that the disease developed when the infested soil was diluted 800 times. From the stand point of practical control, it is important to determine the details on the development of the tobacco stunt disease.

EXPERIMENTAL

1. Relation of position of the intested soil to development of the tobacco stunt disease

In a wooden box 25 cm in length, 25 cm width and 25 cm depth with holes for drainage in the bottom, gravels were spread at the bottom and 2 cm layers of the infested soil was put between sterilized soil as shown in Figure 1. As shown in Table 1, in the section where the 2 cm of the infested soil was put as a top layer in the four experiments carried out between 1952 and 1954, there were between 26.0 to 51.4 percent development in the disease; while in the section where the infested soil layer was put at the position 2 to 4 cm from the surface, during the same period there was only one infected plant. Where the distance of the infested soil from the surface was greater, no plant was infected by the disease.

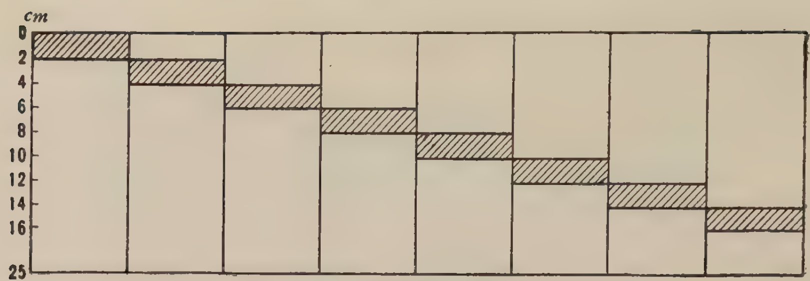


Figure 1. Position of the infested soil

Examples: Infested soil

Sterilized soil

TABLE 1. *Position of the infested soil in relation to development of the disease*

			Position of infested soil (depth—*cm*)							
			0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16
A.	Feb.-May,	No. of plants	47	47	47	47	47	47	47	47
	1952	No. of diseased plants	22	0	0	0	0	0	0	0
B.	Sept.-Nov.,	No. of plants	35	47	47	47	47	47	47	47
	1952	No. of diseased plants	18	0	0	0	0	0	0	0
C.	Sept.-Nov.,	No. of plants	111	77	82	83	73			
	1953	No. of diseased plants	40	1	0	0	0			
D.	Feb.-May,	No. of plants	146	147	144	187				
	1954	No. of diseased plants	38	0	0	0				
		Those with necrosis at soil level	59	12	0	0				

Remark: Plants experimented were "Bright Yellow" variety as in the following tables.

2. *Relation of distribution of the diseased plants to distance from the infested soil in the seedbed*

Experiment I. (March to May, 1953)

As experimented material, the surface soil of the seedbed where the tobacco stunt disease developed in the previous autumn was used. The experiment treatments were arranged as shown in Figure 2, i.e., the infested soil at the width of 10.5 cm and the depth of 3 cm was put in rows, and between these rows sterilized soil at the width of 25.5 cm was put in. Seeds were sown 1st of March, 1953, and the results on the growth were examined on 4th of May; (1) on the infested soil, (2) at the distance of 1.5 cm from

the infested soil, (3) at the distance of 6 cm from the infested soil, and (4) at the distance of 10.5 cm from the infested soil.

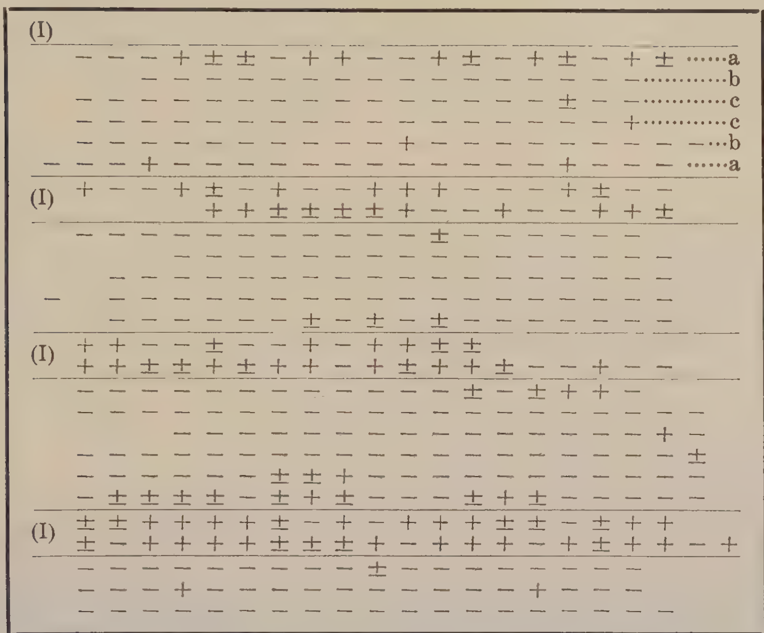


Figure 2. Distribution of the diseased plants in relation to distance from the infested soil in the seedbed

Examples: (I) Infested soil

a, 1.5 cm from infested soil — +, those with typical symptoms

b, 6.0 cm from infested soil — ±, with only soil level necrosis

c, 10.5 cm from infested soil — −, with no symptoms

TABLE 2. *Distance from the infested soil and development of the disease*

(March to May, 1953)

Distance from infested soil	Those with necrosis at the soil level		Those with symptoms above the ground		No. of plants examined
(*cm*)	No. of plants	Percentage	No. of plants	Percentage	
1.5	20	15.2	12	9.1	132
6.0	2	1.5	4	3.0	130
10.5	2	1.8	2	1.8	111
On infested soil	26	24.5	50	47.2	106

Experiment II. (February to May, 1954)

Seeds were sown on steam-sterilized surface soil in the seedbed. By the time the seeds started sprouting (8th March), the surface soil of 7.2 cm×7.2 cm×4.5 cm was dug out at the established intervals and replaced with the same amount of infested soil, taking care not to mix it with other parts of the seedbed. The development was examined on 1st of May.

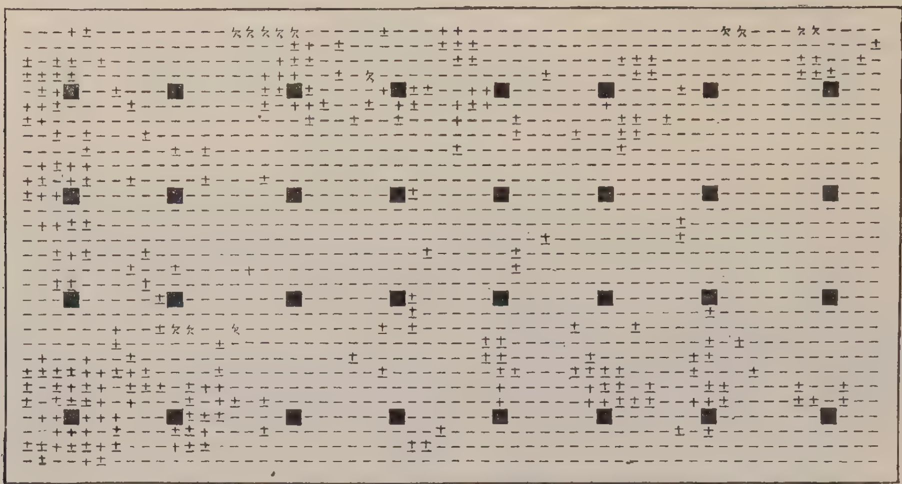


Figure 3. Distribution of diseased plants with reference to the distance from the infested soil

Examples: ■, Infested soil
+, Those with typical symptoms
±, With only soil level necrosis
−, With no symptoms
欠, No plant at examination

TABLE 3. *Distance from the infested soil on the seedbed and the disease development*

(February to May, 1954)

Distance from infested soil	Those with nerosis at the soil level		Those with symptoms above the ground		No. of plants examined
(*cm*)	No. of plants	Percentage	No. of plants	Percentage	
1.5	18	8.1	14	6.3	222
6.6	43	9.9	19	4.4	435
11.7	50	7.8	9	1.4	638
On infested soil	39	21.1	48	25.9	185

The results of the experiments of 1953 are as shown in Table 2 and Figure 2, which shows that the development of the disease is most remarkable on the infested soil, and the greater the distance from the infested soil, the less it becames. If the seeds were sown 6.0 cm or 9.0 cm from the infested soil, the diseased plant was seldom seen. As is seen from Figure 2, the percentage diseased was 9.1 percent at 1.5 cm from the infested soil, 3.0 percent at 6.0 cm distance and 1.8 percent at 10.5 cm distance, thus decreasing with the distance.

Further, the results of the experiment carried out in 1954, as shown in Table 3 and Figure 3, reveal that development of the tobacco stunt disease is most conspicuous around the infested soil. Table 3 shows total of the results of the experiment on distance from the infested soil in relation to percentage diseased. As was seen from the experiment of the previous year, the development decreases with increase in the distance from the infested soil, thus 6.3 percent at 1.5 cm distance, 4.4 percent at 6.6 cm, 1.4 percent at 11.7 cm.

3. Relation between the disease development in the seedbed and in the field.

Experiment I. (May to June, 1953)

In spring, 1953, we picked up from an infested seedbed (diseased plants—40 percent) the seedlings with the disease symptoms and those without; and also from a sterilized seedbed (diseased plants—3 percent) seedlings without symptoms and transplanted them at random in a field.

TABLE 4. *Cases of disease in field seedlings with or without symptoms*

(May to June, 1953)

Kinds of seedlings	No. of plants	No. of diseased plants	Percentage diseased
Seedlings with symptoms	69	69	100.0
Seedlings without symptoms from seedbed infested 40 percent	143	51	35.7
Seedlings without symptoms from seedbed infested 3 percent	71	18	25.4

Above table show that 35.7 percent of the seedlings seemingly healthy from 40 percent infested seedbed later developed the symptoms, and that even the seedlings apparently healthy from the seedbed with only 3 percent infection developed the symptoms on the field later to an extent of 25.4 percent. The fact that even from the seedbed steam-sterilized we found 3 per-

cent development of the disease means probably the sterilization itself was very imperfect, or else after the sterilization unsterilized soil was mixed. This is perhaps a peculiar case and could not be considered as a sample sterilized seedbed. This seedbed would safer be considered as a bed where the percentage diseased was small.

Experiment II. (May to June, 1953)

The seedlings examined here are 69 seedlings of those diseased on the seedbed explained in Experiment I under Chapter 2 above, and 24 of seemingly healthy seedlings each at the distance of 1.5 cm, 6.0 cm and 10.5 cm from the infested soil. We transplanted these to the field (4th May) and examined the results on 21st June.

TABLE 5. *Plants diseased at the field and distance from the infested soil*

(May to June, 1953)

Distance from infested soil in seedbed (*cm*)	No. of plants	No. of plants diseased	Percentage diseased
1.5	24	5	20.8
6.0	24	3	12.5
10.5	24	5	20.8
Diseased seedlings	69	69	100.0

This table shows that the distance from infested soil does not have too great a bearing on the percentage diseased in the field, so that it may be concluded that the distance from the infested soil does not necessarily decide the degree of contamination. This fact can be induced from Figure 3, too. In other words, the soil itself is not evenly contaminated, but if there is any particular part which is highly contaminated, a plant growing at some distance from the spot can be infected, and develop the symptoms later in the fields. (see Figure 2).

Experiment III. (May to July, 1954)

The diseased seedlings in the seedbed shown in Experiment II under Chapter 2 above, and those seemingly healthy ones at the distance each of 1.5 cm, 6.6 cm and 11.7 cm the diseased seedlings were transplanted into the field and were later (9th July) examined as to the disease development.

TABLE 6. *The disease development in the field and the distance from the diseased seedlings in the seedbed*

(May to July, 1954)

Kinds of seedlings (*cm*)	No. of plants	No. of diseased plants	Ill-grown without symptoms	Healthy plants
Those with symptoms	34	28	4	2
Healthy ones at 1.5 distance	68	6	27	35
Healthy ones at 6.6 distance	45	2	17	26
Healthy ones at 11.7 distance	47	10	6	31

Table 6 shows that even the seedlings showing symptoms of the disease in the seedbed masked further symptoms in the field though their growth was poor. Of these two plants showed normal growth. In this experiment we comfirmed that the disease development or the growth of the seedlings after transplanting did not depend much on the distance from the infected seedlings.

Experiment IV. (May to July, 1954)

In Sakai, Nakai-mura, Tokawa, Kitahatano-mura, and Chimura, Nishihatano-cho, Kanagawa Prefecture, seedlings next to the diseased plant and another second from the diseased plant, all seemingly healthy, were collected from infested seedbeds, and transplanted for observation. The growth was recorded on 9th July.

TABLE 7. *The disease development in the field and the distance from diseased seedlings in the seedbed*

(May to July, 1954)

Kinds of seedlings	No. of plants	With remarkable symptoms	With symptoms	Ill-grown without symptoms	Healthy plants
Healthy seedlings next to diseased one	109	9	15	28	57
Healthy seedlings second from diseased one	116	11	19	39	47

Above table shows no difference regarding the percentage diseased between those next to the diseased seedlings and those second from the diseased seedlings after transplanting to the field.

Experiment V. (April to June, 1955)

On 27 the April, 1955, in Tokawa and Bodai, Kitahatano-mura, Kanagawa Prefecture, from an infested seedbed we collected seedlings: A)

next to the diseased ones, B) second from the diseased ones, and C) third from the diseased ones, all seemingly healthy and transplanted in the field for examination.

TABLE 8. *The disease development in the field and the distance from diseased seedlings in the seedbed*

(April to June, 1955)

Distance	No. of plants	No. of diseased plants	Percentage diseased
A	96	15	15.6
B	96	7	7.3
C	96	3	3.1

Above table shows difference between these section (P=0.05), and the fact that the more the distance from diseased seedlings on seedbeds, the less the development in the field.

4. *Dilution by sterilized soil of the infested soil with regards to development of the disease*

The mixture of infested soil with steam-sterilized surface soil (prepared by mixing farmyard manure with the same amount of fresh soil) prepared in the ratio shown below was put in 10 in. pots, and seeds were sown on 22nd September, 1953. Some of the plants started to show the symptoms about 28th October, and the results were examined on 20th November.

TABLE 9. *Dilution of the infested soil and development of the disease*

(September to November, 1953)

Infested soil versus sterilized soil	No. of plants	No. of diseased plants	Percentage diseased
1:0	87	52	59.8
1:1	94	58	61.7
1:3	109	56	51.4
1:7	96	35	36.5
1:15	96	40	41.7
1:31	124	42	33.9
1:63	122	28	23.0
1:127	136	12	8.8

Remark: Above figures show the total results gained from three 10 in. pots each.

Above table shows that the disease development does decrease by dilution of the infested soil, but that it still develops on the soil diluted 127 times.

Discussion

The tobacco stunt disease developed in high percentage only in the section where seeds were sown on the seedbed with infested soil layer 2 cm from the surface of the bed. When the infested soil was placed at lower layer covered with sterilized soil, no disease case was seen. Same results were published by IKATA and KAWAI (1940) of their experiments on wheat yellow mosaic regarding its soil borne character. They explained that when the infested soil was put more than 15 cm deep from the seed sown, no disease was seen, and the shallower the infested soil layer, the more cases were seen. This is perhaps due to the fact that wheat yellow mosaic infects the roots of the plants. The same explanation will perhaps apply to the case of the tobacco stunt disease. The development of the disease will have much to do with the part of the plant where the virus infects and also with the growth stage of the plants.

When we put infested soil exclusively in a certain part of a seedbed by way of examining the development situation of plants thereon, we found out that the greater the distance from the infested soil, the less the disease cases, and the third plants from infected ones (the distance from infested soil being about 10.5 to 11.7 cm were 1.4 to 1.8 percent in percentage diseased. It may safely be said that, therefore, the movement of tobacco stunt virus or its vector in the soil is not too rapid. The contamination of this degree may easily spread with water or other means at the time of seedbed preparation and maintenance, and accordingly, it can be concluded that the development of the tobacco stunt disease in the seedbed scatteringly is due to imperfect sterilization of the surface soil used, or else to the contamination of the whole seedbed during surface soil transportation. The possibility is very small of the virus being rapidly scattered about to cover the whole seedbed from the small infested soil.

The experiments of transplanting seemingly healthy seedlings around the infected ones from the seedbeds to the field, arranging them according to the distance from the infected ones, revealed in one case that the disease development and the growth of th plants did not depend on the difference of distance from the infected ones, and in two other cases that they did depend on it, though situation differed according to the degree of contamination of the seedbed involved. So far, we have not had enough number of experiments, and especially as it can be distributed apart from the infested soil as shown in Figure 3, it is necessary to carry out more experiments with increased number of cases to be examined.

Rather high percentage of the development was seen in the case of

infested soil diluted with steam-sterilized soil up to 63 times, but the more the degree of dilution, the less percentage. The fact that even on the soil 127 times diluted with sterilized soil the disease was seen and tends to make us believe that the disease originates in contaminated soil and that great danger of infection lies in the sterilized soil being mixed with the infested soil.

Summary

The writers have studied the relations between dilution of infested soil with sterilized soil, position and distance from the infested soil in the seedbed to the development of the tobacco stunt disease.

1. A 2 cm layer of infested soil on the upper-most surface was sufficient to cause high percentage of the disease, and layers deeper than 2 cm in depth did not show any infectivity.

2. The development of the diseased plant decreased as it receded from the infested soil in the seedbed. There was the risk of infection for the plant near the infected one although they look perfectly healthy.

3. The disease was found to develop even in the soil diluted 127 times with the sterilized soil.

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STUDIES ON THE TOBACCO STUNT DISEASE

VII. RELATIONS OF SPECIES AND VARIETIES OF *NICOTIANA* AND STAGES OF TOBACCO PLANT TO DEVELOPMENT OF THE TOBACCO STUNT DISEASE

By Zyun HIDAKA, Tetsuo UOZUMI and Chuji HIRUKI

INTRODUCTION

The tobacco stunt disease first became in bright tobacco districts. In recent years, as the bright tobacco took over the domestic varieties, the disease spread to other areas, where it was not known before. In relation to the stage of plant, the disease develops about 40 to 60 days after seeds are sown. In other words, the indications are not seen until the seedlings are ready for transplanting, thus it is a serious problem for the cultivators. After transplanting, however, no infection is seen. It is considered very important from preventive point of view to know of the disease development to the tobacco varieties and also to the plant stages.

EXPERIMENTAL

1. *Relation of species and varieties of Nicotiana to development of the tobacco stunt disease*

During the period of 1953 and 1955, we sowed, on the infested soil in seedbed, seeds of 28 tobacco varieties, 12 wild *Nicotiana* species and tomato, egg-plant and petunia and examined for any indication of the disease on them. Those which did not show any symptom were transplanted in the fields for further examination.

Table 1 shows that, of the 28 varieties of tobacco plants, those diseased were Bright Yellow, Delcrest, Judy's Pride, Gold Dollar, KY 16, KY 35, Kelley, RK 70 and Vamorr 50. Besides these some indications were seen on Oxford 2, Oxford 26, Ibusuki and Kirigasaku. Of the species of which there were a few infected plants, each plant was some tranplanted to a 5 in. pot and kept in an air-conditioned green-house held at a temperature of 17°C for about two months for examination. On these varieties when the necrotic leaves developed waves and spots in the seedbed die away, unlike Bright Yellow variety the new growth always show normal developmnt. There were small necrotic spots on few basal leaves of Xanthi variety. This variety was examined likewise held at 17°C, but did not show further symp-

toms. Dixie Bright 101 is a variety which shows necrotic spots even on healthy soil, and in this experiment the plant sown on infested soil indicated very conspicuous necrotic spots which connected themselves in the shape of waves. Some of their symptoms were difficult to discriminate from those of the tobacco stunt disease. These symptoms were seen at 17°C, though the stunting indication was not shown. Out of 24 diseased seedlings transplanted to the field, three made very poor grown. Whether this is due to the infection of the tobacco stunt disease, it is yet to be cofirmed by grafting experiments. Of the domestic varieties, Ibusuki and Kirigasaku were the only ones of that became diseased. Wild tobacco plants and the three species of Solanaceous plants, egg-plant, tomato and petunia, were all free from symptoms.

TABLE 1. *Plants showing disease symptoms by soil infection of the tobacco stunt disease*

Species or varieties	Experiment dates Sp. 1953	Au. 1953	Sp. 1954	Sp. 1955
Nicotina tabacum L.				
Bright Yellow	+	+	+	+
Oxford 1		—	—	
Oxford 2				±
Oxford 26				±
Dixie Bright 101				±
Dixie Bright 102	—	—	—	
Delcrest				+
Judy's Pride		+	+	+
Vesta 55		—		
Gold Dollar		+		
KY 16		+	+	
KY 35		+	+	
Kelley		+		
RK 70	+	+	+	
Vamorr 50		+	+	
Xanthi				—
Awasenmai	—	—	—	
Enshu		—	—	
Hatano	—	—	—	—
Hatanodaruma	—	—		
Daruma	—			

Species or varieties	Experiment dates Sp. 1953	Au. 1953	Sp. 1954	Sp. 1955
Odaruma				—
Ibusuki				±
Izumi				—
Maruba	—	—		
Kirigasaku				±
Matsukawa	—	—	—	—
Nambu		—		
N. alata				—
N. glauca				—
N. glutinosa	—	—	—	—
N. paniculata				—
N. logiflora				—
N. repanda	—	—	±	—
N. rustica				—
N. gossei				—
N. sanderae				—
N. suaveolens				—
N. trigonophila				—
N. sylvestris	—	—	—	—
Licopersicum esculentum				—
Solanum melongena				—
Petunia hybrida				—

Remarks: +, Those with the tobacco stunt disease symptoms
±, Symptoms were not definite and diseased plant few in number
—, Those with no symptoms

TABLE 2. *Difference in development of the tobacco stunt disease by soil transmission according to tobacco varieties*

Year experiment	Varieties	No. of plants	No. of diseased plants	Percentage diseased
1953	Bright Yellow	102	47	46.1
1955	Dixie Bright 101	146	27	18.5
1953	Dixie Bright 102	106	0	0.0
1955	Delcrest	144	73	50.7
1953	Gold Dollar	97	61	62.9
1953	Judy's Pride	108	46	42.6
1953	KY 16	100	47	47.0
1953	KY 35	93	55	59.1
1953	Kelley	93	56	60.2
1953	Oxford 1	102	0	0.0
1955	Oxford 2	144	1	0.7
1955	Oxford 26	133	10	7.4
1953	RK 70	100	38	38.0
1953	Vesta 55	92	0	0.0
1953	Vamorr 50	78	44	56.4
1955	Xanthi	144	0	0.0
1955	Hatano	144	0	0.0
1953	Hatanodaruma	104	0	0.0
1953	Enshu	102	0	0.0
1953	Maruha	100	0	0.0
1953	Nambu	100	0	0.0
1955	Matsukawa	144	0	0.0
1953	Awasenmai	104	0	0.0
1955	Odaruma	144	0	0.0
1955	Ibusuki	143	1	0.7
1955	Izumi	144	0	0.0
1955	Kirigasaku	144	3	2.1

2. *Relation of stages of tobacco plants to development of the tobacco stunt disease*

Tobacco plants in various stages of growth were transplanted from sterilized surface soil to an infested soil to examine the relation of the plant stages to infection. At transplanting, record on the number of leaves including the cotyledons were taken to show the size of each plant. The experiments were repeated three times from 1952 to 1953. The results of these experiments are shown in Tables 3, 4 and 5.

According to the results, as shown in Table 3, the seedlings 30 days or more old after sowing with 6 to 7 leaves including the cotyledons or larger will not be infected by being transplanted into an infested soil.

TABLE 3. *Plant stages and the disease development* (Sept. to Nov., 1952)

No. of days between sowing and transplanting	No. of leaves at transplanting	No. of plants	No. of disdased plants	Percentage diseased
Sown on infested soil	—	55	31	56.4
20 days	4–5	50	34	68.0
30 days	6–7	51	0	0.0
40 days	8–9	46	0	0.0

Remarks: Plants examined were Bright Yellow variety. Same in the following tables. The number of leaves includes cotyledons. Figure shown are the totals of three 10 in. pots.

TABLE 4. *Plant stages and the disease development* (Feb. to May, 1953)

Sowing dates	No. of days between sowing and transplanting	No. of leaves at transplanting	No. of plants	Those with symptoms		Those with necrosis at soil level	
				No. of plants	Percentage	No. of plants	Percentage
Feb. 2	38	5	100	76	76.0	3	3.0
Feb. 4	36	5	100	67	67.0	10	10.0
Feb. 6	34	4	99	80	80.8	2	2.0
Feb. 8	32	4	100	45	45.0	33	33.0
Feb. 10	30	4	96	47	49.0	16	16.7
Feb. 12	28	4	100	65	65.0	19	19.0
Feb. 14	26	3	100	70	70.0	8	8.0
Feb. 16	24	3	97	56	57.7	9	9.3
Feb. 18	22	3	100	65	65.0	4	4.0
Feb. 20	20	3	100	72	72.0	7	7.0
Feb. 22	18	2	99	54	54.5	14	14.1
Feb. 24	16	2	100	67	67.0	4	4.0

Remark: The number of leaves includes cotyledons.

Table 4 reveals that the seedlings transplanted into infested soil 16 to 38 days after sowing show 45.0 to 80.8 percent, diseased plants, but there was no definite line between the number of days from sowing to trnasplanting and the percentage diseased. This was perhaps because the plants were still susceptible to the disease, despite the number of days passed, for the seeds were sown from the first towards the latter part of February when it was still fairly cold and not favourable for the growth of tobacco plants, and all the plants had no more than 5 leaves at the time of transplanting. The results recorded on Table 5 show that the seedlings transplanted on the infested soil 12 to 29 days after sowing developed the disease. All these plants had 5 to 6 leaves or less. Those directly sown on the infested soil

TABLE 5. *Plant stages and the disease development*
(Sept. to Nov., 1953)

Sowing dates	Trans-planting dates	No. of days between sowing and transplanting	No. of leaves at trans-planting	No. of plants	Those with symptoms		Those with necrosis at soil level	
					No. of plants	percen-tage	No. of plants	percen-tage
Sept. 2	Oct. 8	37	8	74	0	0.0	0	0.0
Sept. 2	Oct. 3	32	7	71	0	0.0	0	0.0
Sept. 2	Sept. 30	29	6	74	1	1.4	0	0.0
Sept. 9	Oct. 2	24	5	73	7	9.6	18	24.5
Sept. 9	Sept. 30	22	4	73	7	9.6	5	6.8
Sept. 19	Oct. 6	18	3	65	25	38.4	11	17.0
Sept. 16	direct	—	—	72	45	62.5	12	16.7

Remark: The number of leaves includes cotyledons.

developed the disease 62.5 percent. The more days from sowing to transplanting, the less the percentage diseased. We can conclude that the seedlings about 30 days old or younger having 4 to 5 leaves including cotyledons are susceptible, and the larger plants do not develop the disease even when transplanted on the infested soil.

DISCUSSION

The susceptibility to the tobacco stunt diesase varies with the varieties of tobacco, and it is rare with domestic species. Of the 28 varieties of tobacco plants among bright tobacco, Vesta 55 was quite free from infection. Oxford strains and Dixie Bright strains were also fairly resistant against the disease. No development was seen in wild tobacco plants, tomato, egg-plant or petunia. It was most seen, however, with Bright Yellow variety which at present occupies greatest area, and the percentage diseased was also high with White Burley. In the case of the tobacco stunt disease, the percentage diseased differs with the variety of tobacco plants. While with tobacco necrosis virus it is found at the roots of some plants though sometimes no symptoms are seen above the ground (SMITH 1935, 1937), no similar fact has been found on the tobacco stunt disease so far. According to Tables 3 and 4, all the seedlings within about 30 days of seed sowing and having 4 to 5 leaves including cotyledons are susceptible to the disease. Larger one even by transplanting on to the infested soil will not develop the disease. Generally, seedlings with about 8 leaves including cotyledons start showing the disease indications on the seedbeds before being transplanted to the fields. It is considered that these seldom get infected immediately before showing the symptoms, but earlier at least

when the plants have about 4 to 5 leaves. It is supposed that tobacco plants are susceptible when they are young with about 4 to 5 leaves or less. But sometimes seedlings with 13 to 14 leaves with no symptoms do develop the disease later on the field. It is necessary, therefore, to investigate into the incubation period after infection through the soil, that is, the period from infection to symptom development, and also into the infection time.

Summary

The paper deals with the relations of species and varieties of *Nicotiana* and plant stage of tobacco to the development of the tobacco stunt disease when the disease was transmitted by infested soil.

1. The typical symptoms of the disease developed in 9 varieties, Bright Yellow, Delcrest, Judy's Pride, Gold Dollar, KY 16, Kelley, RK 70, and Vamorr 50, of 28 tobacco varieties experimented, and these varieties showed high percentages of infection. On the other hand, there was no symptoms of infection in the 12 species of *Nicotiana* (except *N. tabacum*), 10 domestic varieties of tobacco, tomato, egg-plant and petunia.

2. The disease was found to develop on the plant up to the stage of 4 to 5 leaves including cotyledons, when it was planted in the infested soil. No development of the disease was recognized on the plant further than the above stage.

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STUDIES ON THE TOBACCO STUNT DISEASE

VIII. EFFECTS OF SOME TREATMENTS OF INFESTED SOIL ON THE DEVELOPMENT OF THE TOBACCO STUNT DISEASE

By Zyun HIDAKA and Chuji HIRUKI

INTRODUCTION

Presuming from the fact that the infectivity of the infested soil does not decrease for at least four years as explained in Report V, the pathogenes of the tobacco stunt disease should exist in the soil in very stable condition. In order to find out more on the mechanism of the soil borne disease, the infested soil was put into water and examined the infectivity of each layer of sediment in the water, the soil suspension filtered through sieves of different meshes, the suspension according to lengths of time of stabilization and the soil according to length of time of immersion. Examination was also made on the effects of carbon dioxide and oxygen treatments of the infested soil on the infectivity of the disease.

EXPERIMENTAL

1. *Relation of infectivity of each layer of sediment in water to the development of the disease.*

Experiment I. (September to November, 1953)

The infested soil was treated in the way shown in Figure 1, and divided into suspension, floating and deposit. Each of these was added to steam-sterilized surface soil to examine the relation to the infectivity. The primary fractionation was done as indicated "A", and each fraction was further fractionated as shown in "B". The floating material, suspension and deposit gained through these fractionations were taken separately, and the floating material and the deposit were put evenly on the top layer of the pot soil, and the suspension was poured on the surface of the pot soil. The soil poured with the suspension became caked at the surface. So at the time of thinning out the seedling, the soil was lightly tilled. The amount of soil used for the experiment was approximately 1.5 litres (about 1,000 g.) per 10 in. pot. Seeds were sown on 18th September and the results were examined on 26th November. The degree of development of the tobacco stunt disease in each fraction are as shown in Table 1.

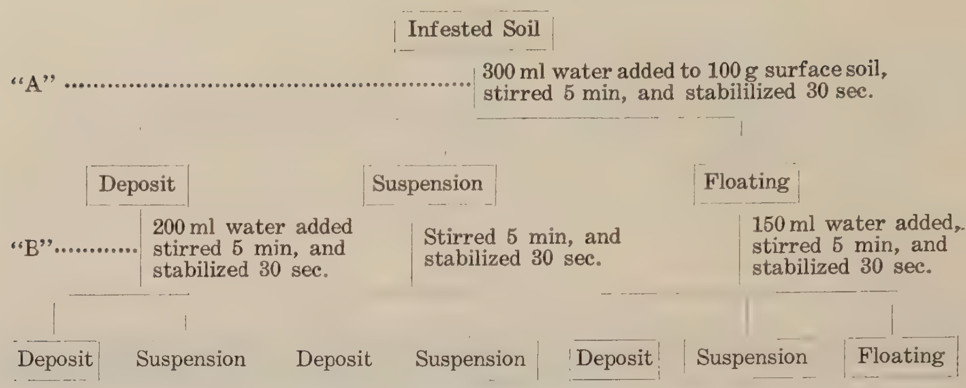


Figure 1. Infested soil fractionation

TABLE 1. *Sedimenting layer of the infested soil in water and the disease development*

	No. of plants	No. of diseased plants	Percentage diseased
Floating	73	13	17.8
Suspension	104	40	38.5
Deposit	29	5	17.3

Remarks: Above figures are the total results gained from three 10 in. pots. Variety of tobacco plants examined is Bright Yellow. Same with the following experiments.

Experiment II. (March to April, 1953)

Test material was from the surface soil collected at Toge, Nishihatano-cho, Kanagawa Prefecture, and of this experiment station where the disease had been seen in the spring and autumn of 1954 respectively. These soil samples were mixed uniformly dried in the shade for two weeks, then sifted through a 3 mm mesh sieve. The amount of infested soil used for the fractionation was 9 litres and for the control 3 litres of unfractionated soil was used. Fractionation was done in the following way.

Suspension : Every litre of infested soil was mixed with twice the amount of water, stirred, floatings taken out, and then filtered through gauze.

Floating : Washed further with water until the filtered water was clear. (Five times with 2 litres water.)

Deposit : Repeatedly washed. (Five times with 2 litres water.)

The material thus treated was added to steam-sterilized surface soil, and seeds were sown on 8th March, and the results were examined on 25th April. They are as shown in Table 2.

TABLE 2. *Sedimented layers of the infested soil in water and the diseased development*

	No. of plants	No. of diseased plants	Percentage diseased
Floated material	156	43	27.6
Suspension	163	100	61.4
Deposit	153	21	13.7
Untreated	171	56	32.7

Remark: Above results are those gained from three 10 in. pots.

These two experiments I (Table 1), and II (Table 2), show that sterilized soil poured with suspension shows high percentage of development, and from the floated material and deposit, after repeated washing, still some disease development was seen.

2. *Relation of filtered suspension of the infested soil to development of the disease*

Experiment I. (September to November, 1953)

The infested soil mixed and stirred with twice the amount of water was aspirated and filtered through a Zeit's aspirater-filter, 6 cm in diametre, with 30, 50, 70, 100, 150, 200 and 250 mesh metal sieves, and a Büchner funnel with gauze or filter paper. The filterates were added to steam-sterilized soil in 10 in. pots. The amount of infested soil used for each was 1 litre.

TABLE 3. *The infested soil filtered and the disease development*

	Width of mesh except wire size (*mm*)	No. of plants	No. of diseased plants	Percentage diseased
30 *mesh*	0.646	103	8	7.8
50 *mesh*	0.361	108	8	7.4
70 *mesh*	0.247	117	3	2.6
100 *mesh*	0.209	98	1	1.0
150 *mesh*	0.171	94	1	1.1
200 *mesh*	0.125	125	1	0.8
250 *mesh*	0.087	120	1	0.8
Gauze		125	3	2.4
Filter paper		111	0	0.0
Infested soil		101	25	24.8

Remark: The above figures are the results gained from three 10 in. pots.

Experiment II. (February to May, 1955)

The experiment material was prepared from the surface soil of the

seedbed where the disease had been seen the previous year, collected and kept in a covered earthen pipe. This was left to dry in the shade for two weeks before the experiment, and filtered through a 3 mm sieve to make it into even clods.

The soil prepared thus was mixed and stirred with twice the amount of water and filtered, stirring all the while with a glass rod so as not to make a mud layer, with a Toyo's sterilizing-filter with 50, 100, 200 and 300 mesh metal sieves. The filtrate thus gained was each divided into five parts. Two litres was mixed with steam-sterilized soil in 10 in. pots, and seeds were sown on them.

TABLE 4. *The infested soil suspension filtered and the disease development*

	No. of plants	No. of diseased plants	Percentage diseased
Gauze	247	221	89.5
50 *mesh*	269	230	85.5
100 *mesh*	228	215	94.3
200 *mesh*	212	180	84.9
300 *mesh*	254	246	96.9
Untreated	220	214	97.3

Remarks: Above figures are total results of five 10 in. pots.
All show high development percentages. We saw 100 percent development in some of the pots of which the suspension was treated with 300 mesh filters.

In Experiment I (Table 3) disease development was seen on the suspension filtered through gauze and 30 to 250 mesh sieves, but the percentage was low.

In Experiment II (Table 4) there was no difference between untreated and treated with gauze and 50 to 300 mesh sieves. The difference in the results gained from these two experiment was perhaps due to the difference in the way of preparation of the material soil and also in the method of filtering.

3. Relation length of time in sedimenting the infested soil to development of the disease

The infested soil added to twice the amount of water was filtered through gauze. Two litres of the filtered suspension was put in a beaker. The suspension was stood at following each time after stirring at room temperature; immediately, 5 min., 30 min., 1 hr., 6 hr., 12 hr., 24 hr., 48 hr., 72 hr., and 96 hr. The each suspension was poured on steam-sterilized soil

each three 10 in. pots and seeds were sown on them.

TABLE 5. *Length of time in sedimenting of the infested soil suspension and the disease development*

Sedimenting time after stirring	No. of plants	No. of diseased plants	Percentage diseased
Immediately	193	153	79.3
5 *minutes*	190	95	50.0
30 *minutes*	209	92	44.0
1 *hours*	238	74	33.1
6 *hours*	215	62	28.8
12 *hours*	110	49	44.5
24 *hours*	212	48	22.6
48 *hours*	156	33	21.2
72 *hours*	213	65	30.5
96 *hours*	151	48	31.8

Remark: Above figures are total results of three 10 in. pots each.

Table 5 shows that the highest percentage was seen on the suspension used immediately after stirring, and the longer the period of sedimenting, the less the development percentages tended to be.

4. Immersion treatment of the infested soil to development of the disease

The infested soil in a Wagner's pot (Experiment A) and in a 2 litres beaker (Experiment B) were added with twice their amount of water and stirred. These were kept for the established number of days and then content was put in sterilized pots and seeds were sown.

TABLE 6. *Immersion treatment of the infested soil and the disease development*

No. of days immersed	Experiment A. (Sept.-Nov., 1953)			Experiment B. (Feb.-May, 1955)		
	No. of plants	No. of diseased plants	Percentage diseased	No. of plants	No. of diseased plants	Percentage diseased
1	103	52	50.5	—	—	—
2	83	49	59.0	—	—	—
4	133	41	59.0	242	145	59.9
8	112	42	37.5	—	—	—
16	145	60	41.4	234	120	51.7
32	96	28	29.2	243	145	59.7
64	—	—	—	175	34	19.4
Untreated	110	59	53.6	197	140	71.1

Remark: Above figures are total results gained from three 10 in. pots.

As seen from Table 6, 64 days immersion treatment still left infectivity of the soil.

5. *Relation of gas treatment of the infested soil to development of the disease*

Five litres of infested soil was placed in the middle layer of a 28 cm diametre desicator laid with a sterilized cheese cloth. Following treatments were made:

1) O_2 for 20 days 2) O_2 for 40 days 3) CO_2 for 20 days
4) CO_2 for 40 days 5) CO_2–O_2 reciprocal treatment 6) Control

Gas treatment was repeated at 10 days interval. At each time the content was exhausted for 30 minutes by an exhaust pump then filled with O_2 or CO_2 gas. In the control section it was exhausted first and then air was introduced. Ten litres of infested soil was used for each treatment, and 2 litres lots of treated soil making a total of five pots were sown with seeds.

TABLE 7. *Gas treatment of the infested soil and the disease development*

Treatment given	No. of plants	No. of diseased plants	Percentage diseased
O_2 treatment 20 days	173	59	34.1
O_2 treatment 40 days	194	63	33.5
CO_2 treatment 20 days	196	44	22.4
CO_2 treatment 40 days	173	49	28.3
CO_2-O_2 reciprocal treatment	172	39	22.7
Control	185	49	26.5

Remark: Above figures are results gained from five 10 in. pots.

Table 7 shows that O_2 treatment had no effect on the infectivity of the tobacco stunt disease. No significant difference in the percentage diseased plants to which CO_2, and CO_2 and O_2 reciprocal treatments were given and that of the control.

DISCUSSION

HOGGAN and JOHNSON (1936) observed that tobacco mosaic virus (TMV) might persist in soil for periods of a year or more, rate of inactivation was more rapid in some conditions. HIDAKA (1947) reported that TMV in the soil, where the mosaic tobacco plants removed, was inactivated with in the year when the soil was tilled, but the virus might overwinter in untilled soil. As to the inactivating influence of oxygen to the virus in

soil, it was found that aeration evidently increased the rate of inactivation both directly and through its influence on microbial activity, and that the variation in inactivating period was depended largely on oxygen relations (JOHNSON, 1926; JOHNSON and HOGGAN, 1937). In the case of the tobacco stunt disease, however, we could discern no difference in infectivity between the soil treated for 40 days with oxygen and the soil not treated. Repeated experiments in this connection will be necessary, however, it is very interesting to know that the tobacco stunt virus, which is considered more unstable *in vitro* than TMV, has remained infective in stored soil for four years, as was reported in Report V, and that the disease develops only when the infested soil is in the layer near to the surface of the seedbed. These facts, together with the rsults of the investigation explained in Report IX, tend to make us believe that the tobacco stunt virus does not exist with the soil particle itself but within the body of some resistant vector. If there is any vector, it must be something smaller than will pass through a 300-mesh sieve. The infested soil has been added with water, and divided into floatings, suspension and deposit to determine the infectivity of each. It was found as the results of these experiments, that sterilized soil poured with this suspension develop the disease as was the infested soil itself. This verified that the suspension was infective. Various other experiments on the subject of soil suspension followed. As to the results of filtering of this suspension, the infectivity was retained in the material that passed through a 300 mesh sieve. Applying the filter paper filtrates of the infested soil to both steam-sterilized and unsterilized non-infested soil, neither wheat mosaic or wheat yellow mosaic occured, whereas the infested soil residues through which water had been filtered several times no visible decrease in the disease development. From these evidences, it was concluded that wheat mosaic virus and wheat yellow mosaic virus are intimately associated within fine soil particles (WEBB, 1928; IKATA and KAWAI, 1940). In our experiments dealt here, pouring the filter-paper filtrate into sterilized soil the tobacco stunt disease did not develop, but since the data has not been accumulated so many as to bear for discussion, furthermore, during the filtering process described above, the soil packed and sealed tightly on the filter-paper resting in the Bücher funnel, it needs further investigation in detail. In the experiments in which the infested soil suspension was allowed to settle down for various periods and the supernates taken after set time, it was found after examination as to their infectivities that, with lapse of time, the infectivity showed a decreasing tendency. We still have to study the relation between this result and the one obtained by filtering experiments. In this connection, the investigations

on the fractions of infested soil obtained by centrifugal technique are being continued.

Summary

In this report the writers studies effects of various treatments of the infested soil on the development of the tobacco stunt disease.

1. When each of the fractions of the infested soil was tested as to its infectivity after they were fractionated into floating, suspension and deposit, it was found that each fraction retained the same infectivity as the infested soil.

2. The soil suspension filtered through a 300 mesh sieve had the same infectivity as the original infested soil.

3. With the laps of time allowed for the suspension to settle down, its infectivity showed a decreasing tendency.

4. The infested soil was found to retain its infectivity even after 64 days of continuous immersion in water.

5. The infested soil did not show any difference in infectiveness when it was treated with O_2 and CO_2 for 20 days and 40 days respectively, and when it was treated with O_2 and CO_2 for 10 days alternately during the period of 40 days. The fact the treatment with O_2 for 40 days did not reduce its infectivity is considered to be a proof that the causal agent was present in the soil in a stable condition with some mechanisms.

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STUDIES ON THE TOBACCO STUNT DISEASE

IX. EFFECTS OF SOIL TREATMENTS WITH CHEMICALS ON THE DEVELOPMENT OF THE TOBACCO STUNT DISEASE

By Zyun HIDAKA, Chuji HIRUKI and Tetsuo UOZUMI

INTRODUCTION

A large number of experiments have so far been carried out as regards the soil sterilization by way of prevention of soil borne virus diseases. For wheat mosaic and wheat yellow mosaic disease it has been reported that formaline, calcium cyanamide, carbon disulphide, chloropicrin, methyl bromid, naphthalene and derris powder are effective for the purpose (MC-KINNEY, 1923; SAWADA, 1927; IKATA and KAWAI 1940; JOHNSON, 1945), while for lettuce big-vein chloropicrin, and D-D (JAGGER and CHANDLER, 1934; ALLEN, 1945), and for tobacco necrosis formalin is effective (SZIRMAI, 1953). These chemicals, too, are effective on different microorganisms. This fact, it is considered, may offer some clue for the study of the mechanism of soil borne nature. With this purpose in view, experiments have been made on the infested soil and its suspension with the aid of various fungicides and insecticides.

EXPERIMENTAL

Experiment I.

MATERIALS AND METHODS

A) In the ratio shown in Table 1 chemicals in powder form were throughly mixed with the infested soil, and those in suspension were poured on to the infested soil spread over a sheet metal for mixing. These mixtures

TABLE 1. *Methods of chemical treatments of the infested soil*

(February to May, 1953)

Chemicals	Particulas	Amounts of chemicals	Amounts of soil (*kg*)
Usplun	1:800 solution	2 *litres*	5
Carbolic acid	3% solution	2 *litres*	5
DDT	5% powder	20 *g*	5
Pyrethrum powder	Powder	30 *g*	5
Derris powder	Powder	30 *g*	5
Seresan-lime mixed	Powder	25 *g*	5

Remark: Seresan-lime consists of Seresan: lime, 1:4.

were put into sterilized pots. In order to prevent any harmful effect from the chemicals, each pot was watered daily for 10 days, after which seeds were sown (February to May, 1953).

B) To some infested soil in a Wagner's pot, chemical liquid in the amount twice that of soil was added and left for 5 hours. It was then put

TABLE 2. *Chemical treatment of the infested soil and the disease development*

Chemicals	Dilution	Experiments	Year	No. of plants	No. of diseased plants	Percentage diseased
Usplun	1:1000	B	1953	123	40	32.5
	1: 800	A	1953	168	16	9.5
	1: 800	C	1954	254	126	49.6
	1: 400	D	1955	241	67	27.8
	3%	A	1953	149	7	4.7
Carbolic acid	3%	B	1953	137	0	0.0
	3%	C	1954	173	0	0.0
Mercuric chloride	1:1000	B	1953	158	0	0.0
	1:1000	C	1954	118	5	4.2
Formalin	3%	B	1953	146	0	0.0
	3%	C	1954	186	0	0.0
DDT emulsion	1:2000	B	1953	97	35	31.6
	1:2000	C	1954	190	41	21.6
DDT powder		A	1953	146	40	27.4
Derris powder		A	1953	145	42	29.0
Pyrethrum powder		A	1953	142	44	31.0
Pestox	1: 800	D	1955	111	86	77.5
Seresan-lime		A	1953	149	33	22.1
Riogen	1: 400	D	1955	204	89	43.6
Ruberon	1: 400	D	1955	214	7	3.3
Copper sulphate	1: 500	D	1955	133	14	10.5
Chloroform	1: 500	D	1955	307	107	34.9
Salcylic acid	1: 500	D	1955	133	97	73.0
Paranitrophenol	1:4000	D	1955	133	102	76.7
Urethan	1:1000	D	1955	134	96	71.6
Chloropicrin		C	1954	187	0	0.0
Carbon disulphide		C	1954	204	0	0.0
D-D		E	1955	184	0	0.0
Toluol		E	1955	195	94	43.1
Xylol		E	1955	140	43	30.7
		A	1953	139	50	36.0
		B	1953	111	71	64.0
Untreated		C	1954	244	61	25.0
		D	1955	120	68	56.7
		E	1955	178	124	69.7

Remark: Above figures are the total results gained from three 10 in. pots.

into a pot, watered daily for 10 days and then seeds were sown. As a control, the infested soil emmersed in water for 5 hours was examined at the same time (September to November, 1953).

C) Same treatment was given to the infested soil with liquid chemicals as in B. Carbon disulphide and chloropicrin, 2 ml each, were used with 1 litre infested soil in air-tight glass vessels and kept for one week (February to May, 1954).

D) The infested soil was put in an air-tight glass vessel; D-D, toluol and xylol, each 1 ml per litre of the soil, were added and left thus for 19 days. The vessel was opened, the gas was allowed to escape, and the soil was put in pots. Seeds were sown on them (February to May, 1955).

E) The infested soil treated in the same way as in B was put in pots, watered daily for 20 days and seeds were sown on them (February to May, 1955).

Results

According to the results shown in Table 2, the treated soil either did not develop any disease, or was less than 10 percent of the total seedlings in carbolic acid, mercuric chloride, formalin, Ruberon, chloropicrin, carbon disulphide and D-D: copper sulphate was also fairly effective, and Seresan-lime, Usplun and xylol treatments, too, decreased the percentage diseased ($P=0.05$ significant). Riogen, salcylic acid, paranitrophenol, urethan, chloroform and toluol treatments were not significant in effect. Derris powder, pyrethrum powder, pestox and DDT and other insecticides did not seem to have any direct effect on the development of this disease.

Experiment II.

Materials and methods

The infested soil was added with twice the amount of water and the suspension was filtered through gauze. For each chemical, 6 litres was prepared. To this the below-mentioned chemicals were added to establish a concentration, and after 5 hours this was poured on to sterilized soil contained in 10 in. pots, 2 litres each. These pots were watered daily for 20 days and seeds were sown on them.

Results

Table 3 shown that Usplun, Ruberon, mercuric chloride, carbolic acid and formalin completely prevented the disease development, and copper

sulphate, salcylic acid and urethan in the same concentration as in previous experiment showed significant difference in the development of the disease. Chloroform and paranitrophenol were not worth mentioning as was the case with previous experiment. Riogen, urethan and salcylic acid, which did not show effectiveness in the previous experiment, reduced significantly percentage diseased in the same concentration in this experiment.

Discussion

Wheat mosaic and wheat yellow mosaic, which are soil borne in the field, are difficult to prevent except by cultivating the resistant varieties against the diseases, though various experiments have been carried out. In the case of the tobacco stunt disease, however, since this is transmitted while on seedbeds, it is possible to control it completely by steam-sterilization of the seedbed soil. It is practically prevented in this way. But if chemical treatment of the infested soil could have any effect on the disease development, this could give us a clue for the study of the mechanism of its soil borne, though it may not be practically used as control measures. Johnson (1945) reported as the results gained by his experiments using various insecticides and nematocides that these would perhaps have effects

Table 3. *Treatment of suspension of the infested soil with chemicals and the disease development*

(February to May, 1955)

Chemicals	Dilution	No. of plants	No. of diseased plants	Percentage diseased
Usplun	1: 400	170	6	3.5
Riogen	1: 400	112	46	41.1
Ruberon	1: 400	109	0	0.0
Mercuric chloride	1:1000	195	0	0.0
Carbolic acid	3%	177	0	0.0
Formalin	3%	158	0	0.0
Copper sulphate	1: 500	173	1	0.6
Chloroform	1: 500	160	79	49.4
Salcylic acid	1: 500	127	38	29.9
Paranitrophenol	1:4000	129	68	52.7
Urethan	1:1000	126	15	11.9
Unteated		112	67	59.8

Remark: Above figures are the total results gained from three 10 in. pots.

on the vector rather than on the virus itself. Derris powder, pyrethrum powder, DDT, Pestox and other insecticides did not prove themselves effec-

tive in the experiments dealt with here. This would mean that insects in the soil have nothing to do with the tobacco stunt disease. As for virus in the soil, IKATA and KAWAI (1940) explained that the virus for wheat yellow mosaic do not exist in the soil solution, but do exist in the fine soil particles of less than 0.25 mm in size and might penetrate through roots without any vector. VAN DER WANT (1951) suggested a possibility of virus being adsorbed in clay minerals. Further it is also possible that soil borne viruses are related with some of soil microbes besides higher plants (MCKINNEY, 1946; BAWDEN and KASSANIS, 1947; VAN DER WANT, 1951). Carblic acid, mercuric chloride, formalin and others completely prevented development of this disease, but it is difficult to determine if these acted on the virus itself or on its vector by the experiments so far carried out. Of the sterilizers utilized, mercuric compounds are worth mentioning and specially Ruberon showed remarkable effect. Usplun, too, was effective, but Riogen was not good in the soil treatment, though with suspension treated we could reduce the percentage diseased a little. As a general, mercuric compounds are very effective in inorganic compound on bacteria and in organic compound on fungi. Ruberon has ethyl mercuric phosphate as principal ingredient; Usplun, methoxyethyl mercuric chloride; Riogen, pheyl mercuric acetate. All these differ in sterilizing power according to the kind of germs. Summarizing all these facts and the results of the experiments leads us to suppose that some fungi might exist as virus vector for the tobacco stunt disease. The fact that the chemical which was effective in the suspension treatment was not effective in the soil treatment means, perhaps, that the effective ingredient of this chemical was adsorbed in soil particle, or dissolved or, again, there was some part where the chemical did not permeate. The chemicals which were effective in these experiments are not necssarily possible to be used practically as prevention chemicals.

SUMMARY

In order to investigate the mechanism of the transmission by soil of the tobacco stunt disease, the writers have studied the effects of several fungicides and insecticides on the infectivity of infested soil and its water suspension.

1. When the infested soil was treated with carbolic acid, mercuric chloride, formalin, Ruberon, chloropicrin, carbon disulphide, D-D, and copper sulphate, the infested soil did not either develop its infectivity or only up to ten percent. Ceresan, Uspulun, and xylol were not very effective.

2. Riogen salicylic acid and urethan were not effective in treating the infested soil, but they were found to be effective in treating the suspension of the infested soil.

3. Insecticides such as derris powder, pyrethum powder, pestox, and DDT were found to have no direct effect on the development of the disease.

4. It was supposed that there is a possibility for soil microbes to act as a vector of the disease.

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STUDIES ON THE TOBACCO STUNT DISEASE

X. EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF THE TOBACCO STUNT DISEASE

By Zyun HIDAKA and Chuji HIRUKI

INTRODUCTION

Influence of climate factors, especially the temperature, on the occurrence of soil borne virus diseases has been shown in wheat yellow mosaic (IKATA and KAWAI, 1940) and lettuce big vein (THOMPSON and DOOLITTLE, 1942). The authors have reported that the tobacco plants infected with tobacco stunt virus developed strinking severe symptoms at the temperature of 17°C, while the symptoms were masked perfectly at 25°C, making a grew as that of a healthy plant (HIDAKA et al. 1955). The following experiments were carried out in order to investigate the influence of temperature on the development of the tobacco stunt disease after sowing in infested soil.

MATERIALS AND METHODS

Experiment I.

The soil used in these experiments were collected in the spring, 1955, from the surface of the seedbed where the disease had developed the previous year, in Bodai, Kitahatano-mura, Kanagawa Prefecture. The soil was kept in a earthen pipe. Before experiments, it was dried in the shade for two weeks, then in a mortar, using rubber stopper pulverized and made into even clods by sifting through a 3 mm sieve. This was put in 10 in. pots, 200 g each as the top layer. As a control, sterilized soil only was put in two pots for every section of the experiment. On 20th September, tobacco seeds (Bright Yellow variety) were sown and the results were examined on 1st, 11th and 21st November. The experiment sections prepared were; A) 17°C for 60 days, B) 25° for 60 days, C) 17° for 30 days and later at 25° for 30 days, and D) 25° for 30 days and later at 17° for 30 days. Five pots were prepared for each of the above four sections and two pots only sterilized soil were put as a check in each section. For section B and C above, after 60 days of sowing, the pots were left at the temperature of 17° and the results were examined on 12th December which

are shown in Table 1-*2*. All these experiments were carried out in an air-conditioned glasshouse.

Experiment II.

Tobacco plants (Bright Yellow variety) were grown for 30 days in an electrically heated seedbed kept at 25° to 28° with the infested soil as a surface soil. Some seedlings were picked up at random and transplanted onto to 10 in. pots with sterilized soil and then transferred to an air-conditioned glasshouse for treatment, with exception that the plants of Section D were transplanted onto a seedbed. The sections prepared were; A) 17° for 30 days, B) 25° for 30 days, C) 17 and 25° each other day alternately for 30 days, and D) Electrically heated seedbed for 30 days (soil temperature, 25° to 28°). As a control, sterilized soil was put on a seedbed in the same conditions, and seedlings were transplanted onto pots 30 days after seed sowing, and two pots were put in each section. In the daytime, the seedbeds were kept at 25° to 28°, but at night the temperature often fell below 20°. The temperature was measured at the sub-soil, so it cannot be said to have been very accurate. The disease development was examined for each section three times, i.e., 1st, 11th, and 21st of November. For section B, the examination was done 30 days after transplanting, and then the pots were kept at the temperature of 17° and the results were again examined on 12th December, as shown in Table 2-*2*.

Results

Experiment I.

In Sections A (17° for 60 days) and D (25° for 30 days, and later at 17° for 30 days) plants started showing symptoms in about 35 days after seed sowing (see X-PL. I, Fig. 1 and II, Fig. 1), while in Sections B (25° for 60 days) and C (17° for 30 days, and later at 25° for 30 days) no symptoms of the disease were seen even after 60 days of seed sowing (see X-PL. I, Fig. 2 and X-PL. II, Fig. 2). When the plants of Sections B and C were transferred to the temperature of 17°, they started showing symptoms of the tobacco stunt disease, and with the passing of time top leaves and veins developed necrosis, internodes stopped growing, and the growth was conspicuously suppressed revealing characteristic symptoms of the tobacco stunt disease (see X-PL. III, Figs. 1 and 2). As shown in Table 1-*1*, plants in Section D which were kept at a high temperature, namely at 25°, for 30 days immediately after seed sowing, in comparison with those in Section A which were kept for 30 days in the

low temperature, namely at 17°, indicated low disease percentage even though they were later kept at 17° for 30 days. Further, plants of Section B which were kept at 25° after seed sowing for 60 days and then transferred to 17° and kept for 20 days here showed lower percentages of the disease development compared with those in Section C which were first kept at 17° for 30 days after seed sowing and then at 25° for 30 days and lastly for 20 days at 17°, as shown in Table 1-*2*.

Experiment II.

Table 2-*1* shows that plants in Section A, which were kept at 17° for 30 days after being transplanted from an electrically heated seedbed to sterilized soil, showed some disease development, while those in Section B which were kept at 25° for 30 days showed no disease case. The plants in Section C which were treated at 25° and 17° each on alternate days showed some disease cases, thus showing effects of being kept at low temperature. Section D (transplanting in electrically heated seedbed) also showed high percentage of disease development. When tobacco plants kept at 25° for 30 days and showing healthy growth were put at 17° showed typical symptoms of the tobacco stunt disease, and the disease percentage was rather high as is explained in Table 2-*2*.

Discussion

Jagger and Chandler (1934), Thompson and Doolittle (1942) observed that for the expression of the lettuce big-vein symptoms, it needs low temperatures. The latter reported that the expression of symptoms of this disease is prevented at high temperature (daytime, 65° to 75°F; night, 50° to 60°F.), and the plant showing no evidence of the disease but developed the typical symptoms of the big-vein disease by exposing at lower temperature (daytime, 50° to 60°F; night, 45° to 50°F.) and the glasshouse temperature again raised to the higher level suppressed symptoms. Pryor (1946) reported that when air temperature is favourable for the production of big-vein symptoms, a soil temperature of about 22° is optimum for disease development. Ikata and Kawai (1940) observed that the optimum soil temperature for the development of wheat yellow mosaic was found to be about 15°. It was fairly severe at 10°, while at 20° the symptoms were inconspicuous, and at 25° imperceptible. Since the disease did not occure at 30° they thought wheat did not become infected with the virus at such a high temperature. According to the results of

TABLE 1–*1*. *Effects of temperature on the development of the tobacco stunt disease*

Section	Pot No.	No. of plants	No. of diseased plants I (Nov. 1)	II (Nov. 11)	III (Nov. 21)	Total	Percentage diseased
	1	71	22	15	21	58	81.5
	2	81	18	20	30	68	84.0
A	3	78	13	16	25	54	69.2
	4	65	13	15	17	45	69.2
	5	84	25	22	12	59	70.2
Check	1	85	0	0	0	0	0.0
	2	82	0	0	0	0	0.0
	1	74	0	0	1	1	1.3
	2	39	0	0	1	1	2.6
B	3	47	0	0	0	0	0.0
	4	42	0	0	0	0	0.0
	5	49	0	0	0	0	0.0
Check	1	73	0	0	0	0	0.0
	2	68	0	0	0	0	0.0
	1	72	0	0	0	0	0.0
	2	54	0	0	0	0	0.0
C	3	58	0	0	0	0	0.0
	4	51	0	0	0	0	0.0
	5	63	0	0	0	0	0.0
Check	1	83	0	0	0	0	0.0
	2	86	0	0	0	0	0.0
	1	85	14	14	45	73	85.9
	2	87	2	6	10	18	20.7
D	3	71	3	7	11	21	29.6
	4	86	2	5	13	20	23.2
	5	68	2	9	6	17	25.0
Check	1	73	0	0	0	0	0.0
	2	78	0	0	0	0	0.0

this experiment, the tobacco plants sown on the infested soil showed no symptoms up to the 30th day both at 17° and 25°, but those kept at 17° the first symptoms were seen on the 35th day and others succeeded. Those kept at 17° for 30 days and then transferred to 25° showed no symptoms throughout the period at 25° and grew up seemingly healthy. On the other hand, those kept at 25° for 30 days and then transferred to 17° started showing the symptoms a few days after the transfer. The fact that they did not show any symptoms before the 30th day and started

TABLE 2–1. *Effects of temperature on the development of the tobacco stunt disease*

Section	Pot No.	No. of plants	No. of diseased plants I (Nov. 1)	II (Nov. 11)	III (Nov. 21)	Total	Percentage diseased
	1	51	0	3	8	11	21.6
	2	54	0	3	10	13	24.1
A	3	54	0	2	9	11	20.4
	4	56	0	3	7	10	17.9
	5	54	0	2	11	13	24.1
Check	1	52	0	0	0	0	0.0
	2	55	0	0	0	0	0.0
	1	44	0	0	0	0	0.0
	2	47	0	0	0	0	0.0
B	3	53	0	0	0	0	0.0
	4	52	0	0	0	0	0.0
	5	55	0	0	0	0	0.0
Check	1	54	0	0	0	0	0.0
	2	43	0	0	0	0	0.0
	1	52	0	1	8	9	17.3
	2	52	0	2	9	11	12.2
C	3	51	0	1	8	9	17.6
	4	54	0	3	5	8	14.8
	5	50	0	3	7	10	20.0
Check	1	55	0	0	0	0	0.0
	2	54	0	0	0	0	0.0
	1	50	2	6	11	19	38.0
	2	50	1	8	11	20	40.0
D	3	52	0	8	14	22	42.3
	4	50	1	9	11	21	42.0
	5	51	0	7	12	19	37.3
Check	1	144	0	0	0	0	0.0
	2	144	0	0	0	0	0.0

showing it about the 40th day perhaps indicates that they had been infected with the vrus while they were still at 25°. Every 10 days' examination of those kept at 17° for 60 days and the same examination of those first kept at 25° for 30 days and then at 17° for 30 days show that with the former even rate of increase in the disease development was seen, while with the latter the disase cases increased more rapidly with the lapse of time. In Section D where the plants were kept first at 25° for 30 days and in Section B where they were for 60 days at 25° the disease percent-

TABLE 1–2. *The disease development at 17° after treatment of Sections B and C.*

Section	Pot No.	No. of plants	No. of diseased plants	Percentage diseasep
	1	41	8	19.5
	2	13	6	46.2
B	3	24	5	20.8
	4	18	11	61.1
	5	18	4	22.2
	1	39	36	92.3
	2	41	41	100.0
C	3	36	30	83.3
	4	42	37	88.1
	5	32	25	78.1

TABLE 2–2. *The disease development at 17° after treatment of Section B*

Pot No.	No. of plants	No. of diseased plants	Percentage diseased
1	28	9	32.1
2	31	13	41.9
3	34	13	38.2
4	31	11	35.5
5	33	4	12.1

ages were lower than those in Section C, where the plants were first kept at 17° for 30 days or Section A where they were kept at 17° for 60 days throughout. Since even those in Section B, when transferred at 17°, showed symptoms on the second day, it is probable that those in Sections D and C which were transferred to 17° after 60 days and which developed symptoms later had already been infected within this 60 days period. It is very interesting, however, that there was a difference in the percentages of disease development between Sections D and B on one hand and Sections C and A on the other, though the reasons for this fact are yet to be determined. There is a remarkable difference in the development ratio between 17° and 25°, and those infected while at 17° showed healthy growth at 25°C. It is evident that these had already been infected, for if these were transferred from 25° to 17° they started showing the symptoms in a few days or even on the following day sometimes. In this experiment the difference was seen between the disease development of those kept at 17° for 60 days after seed sowing and of those kept at 25° for 30 days after seed sowing and then at 17° for 30 days, the latter being far lower. When

the plants kept at 25° for 60 days and these first kept at 17° for 30 days and then at 25° for 30 days were transferred to 17°, the disease development percentages were different, the former being lower than the latter. We can therefore summarize that for the development of the tobacco stunt disease the temperature in the vicinity of 17° is the most fabourable, though the infection is possible at 25°, and that development of the disease is closely related with the degree of temperature. It is found that though high temperature does not favour development of the tobacco stunt disease, if lower temperature is inserted in between, the disease can develop.

Summary

This paper reports the effect of temperature on development of the tobacco stunt disease when experimented in the air-conditioned glasshouse.

1. Symptoms of the disease began to develop on the 35th day after sowing tobacco at 17°. It was shown that this temperature was fabourable to produce the symptoms.
2. The symptoms did not develop when the plant was kept at 25 for 30 days, but when it was kept at 25° and 17° each other day alternately, symptoms were found to develop.
3. Symptoms did not develop on the plants kept at 25°, but some of them were found to have been infected.
4. The plant kept at 25° for 60 days or at 25° for 30 days and then 17° for 30 days after sowing developed less infection than the plant kept at 17° for 60 days, or at 17° for 30 days and then at 25° for 30 days.
5. The plant kept at 17° for 30 days and then a 25° for 30 days after sowing did not develop any symptoms, but when treated with 17° temperature again, it developed symptoms.

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EXPLANATION OF X-PL. I

Fig. 1. Sixty days at 17° after seed sowing.

Left: Seed sown on sterilized soil mixed with 200 g infested soil. Typical symptoms of the disease are seen, and the growth is strikingly suppressed.

Right: Control (steam-sterilized soil only).

Fig. 2. Sixty days at 25° after seed sowing.

Left: Seed sown on sterilized soil mixed with 200 g infested soil. No symptoms of the disease. The growth is similar to one on sterilized soil.

Right: Control (steam-sterilized soil only).



Fig. 1



Fig. 2

EXPLANATION OF X-PL. II

Fig. 1. Thirty days at 25° after seed sowing, and then for 30 days at 17°.

Left: Seed sown on sterilized soil mixed with 200 g infested soil. Typical symptoms of the disease seen and the growth strikingly suppressed.

Right: Control (steam-sterilized soil only).

Fig. 2. Thirty days at 17° after seed sowing, and then for 30 days at 25°.

Left: Seed sown on sterilized soil mixed with 200 g infested soil. No symptoms of the disease seen, and the growth is normal.

Right: Control (steam-sterilized soil only).



Fig. 1



Fig. 2

EXPLANATION OF X-PL. III

Fig. 1. Seed was sown on the infested soil. The seedlings were kept at 25° for 60 days, and the plants showed healthy growth. Then they were transferred to 17° and kept there for 20 days. It can be seen as necrotic waves, deformated leaves and mottling.

Fig. 2. Thirty days at 17° and then at 25°. The plant showed seemingly healthy growth. It was then transferred to 17° again and kept for 20 days. Typical disease symptoms are seen here.



Fig. 1

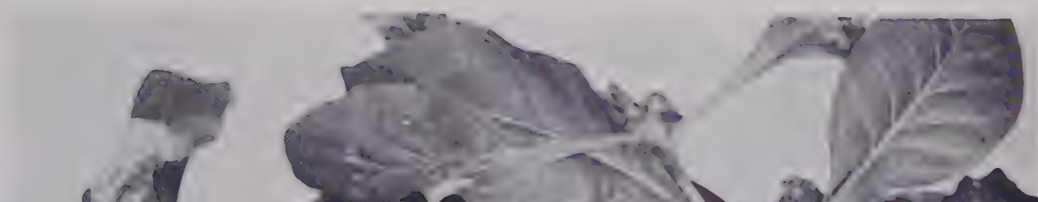


Fig. 2

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STUDIES ON THE TOBACCO STUNT DISEASE

XI. CONTROL OF THE TOBACCO STUNT DISEASE

By Zyun HIDAKA

INTRODUCTION

For the soil sterilization of soil borne virus disease, formalin, chloropicrin, calcium cyanamide and steam sterilization have so far been used for wheat mosaic (MCKINNEY, 1923; JOHNSON, 1945) and wheat yellow mosaic (SAWADA, 1927; IKATA and KAWAI, 1940), chloropicrin, D-D, formalin and steam sterilization for lettuce big-vein (JAGGER and CHANDLER, 1934; ALLEN, 1948), while for tobacco necrosis formalin has been used (SZIRMAI, 1953). These diseases can be prevented by steam sterilization, but chemical treatment is often inadequate. Report I dealt with the findings of the research party and explained that the disease in question could be completely prevented by steam-sterilization of the soil for seedbeds. Steam-sterilization, however, cannot easily be carried out due to the time, labour, implement and fuel required. This report is on the methods of using chemical as a sterilizing agent.

EXPERIMENTAL

Experiment I.

Some surface soil was collected from Mr. S. INOUE's seedbed at Mikurube, Kamihatano-mura, Kanagawa Prefecture, whose seedbed in 1951 had many tobacco stunt disease. The soil was brought to this experiment station and kept in an earthen pipe buried vertically until its use. The experiment consisted of: (1) steam sterilization (for 60 min. at 95°C.), (2) chloropicrin sterilization (1 ml per 1 *to* (about 18 litres) of the infested soil), (3) same 3 ml, (4) same 5 ml, and (5) not sterilized. In chloropicrin sterilization experiment, a 1 : 20,000 Wager's pot was used. In the infested soil contained in this pot, a hole was made in the centre where the above mentioned amount of chloropicrin was poured. The hole was then closed and was covered with four-fold cellophane layers immediately. After 12 days, the soil was put into steam-sterilized 10 in. pots. In the soil treated with 3 ml and 5 ml of chloropicrin the chemical still smelled rather strongly, the growth of seeds or the plant was not hampered in the least. The experiments were carried out during the period of September to November in 1951.

TABLE 1. *Effects of steam and chloropicrin sterilization of the infested soil to development of the tobacco stunt disease*

(September to November, 1951)

	Not treated	Steam-sterilization	Chloropicrin sterilization 1 *ml*	3 *ml*	5 *ml*
No. of plants	404	245	231	236	269
No. of diseased plants	89	0	1	0	0
Percentage diseased	22.0	0.0	0.4	0.0	0.0

Remark: Plants experimented were Bright Yellow variety, as in the cases of the following experiments.

Table 1 shows that steam sterilization is definitely effective, while chloropicrin sterilization, too, shows almost perfectly effect. We may be able to expect almost the same result from the latter as from the former.

Experiment II.

There were numerous cases of this disease in 1949 in the seedbeds belonging to Messrs. SEKINO and YAMAGUCHI at Yokono, Kitahatano-mura, Kanagawa Prefecture. Experiments were made here sterilizing the seedbed soil for 1950 tobacco plants. Chloropicrin in the amount of 10 ml per 1 *koku* (about 180 litres) was poured in the hole made in the pile of the seedbed soil. Steam sterilization was made for 10 minutes with temperature of 90°C. The results are shown in Table 2.

TABLE 2. *Effects of sterilization of the seedbed soil to development of the tobacco stunt disease*

(February to May, 1950)

	Sekino's seedbed			Yamaguchi's seedbed		
	Not treated	Chloropicrin	Steam	Not treated	Chloropicrin	Steam
No. of plants	1,566	1,566	1,566	1,275	1,275	1,275
No. of diseased plants	26	28	8	398	178	67
Percentage diseased	1.7	1.9	0.5	31.2	14.0	5.2

As regards Mr. SEKINO's seedbed, the development of the disease was too few and the results were not reliable. Mr. YAMAGUCHI's cases show that the results of steam sterilization were preferable to those of chloropicrin treated seedbeds, however, though the diseased seedlings were many, they were seen in groups and it was not difficult to pick up healthy seedlings.

Experiment III.

The soil prepared for the seedbed for 1952 at Mikurube, Kamihatano-mura, Kanagawa Prefecture, where there had been many cases of this disease every year, was mixed with same manures (1.5 *sho* (about 2.7 litres) of fermented rape seed cake per *tsubo* (about 3.3 m^2)), and this was used for the surface soil. Soil sterilization was carried out in the following nine different ways: (1) not treated, (2) ethylene bromide 200 ml, (3) same 400 ml, (4) D-D 120 ml, (5) same 240 ml, (6) chloropicrin 40 ml, (7) same 80 ml, (8) same 120 ml, and (9) same 160 ml. The amount of chemicals specified are for 4 *koku*. The soil to be sterilized was put in earthen pipe of approximately 60 cm in diametre held vertically. The results of this experiment are shown in Table 3. Some effects are seen but with others no effect is recognizable.

Experiment IV.

In the seedbeds of Messrs. INOUE, UCHIDA and OSAWA at Mikurube, Kamihatano-mura, Kanagawa Prefecture, effects of the soil sterilization by chloropicrin were examined in relation to development of the tobacco stunt disease. Chloropicrin was used in three different quantities of 40 ml, 80 ml and 120 ml per 4 *koku* of the soil, and additional one none-treatment section was prepared. Sterilization was made on 16th February, and seeds were sown on 25th February.

TABLE 3. *Effects of chemical sterilization of the infested soil to development of the tobacco stunt disease*

(February to May, 1952)

Chemicals and quantity	(*ml*)	No. of plants	No. of diseased plants	Percentage diseased
Not treated		805	585	72.7
Ethylene bromide	200	805	482	59.9
Ethylene bromide	400	805	448	55.7
D–D	120	805	182	22.6
D–D	240	805	51	6.3
Chloropicrin	40	805	464	57.6
Chloropicrin	80	805	362	45.0
Chloropicrin	120	805	247	30.7
Chloropicrin	160	805	227	28.2

Table 4 shows that Mr. INOUE's sterilized seedbed proved some effects while Mr. UCHIDA's failed to show any effect. In Mr. OSAWA's seedbeds stelirized with chloropicrin the diseased seedlings were in groups enabling

TABLE 4. *Effects of chloropicrin sterilization to development of the tobacco stunt disease*

(February to May, 1952)

	Inoue's seedbed				Uchida's seedbed				Osawa's seedbed			
	Not treated	40*ml*	80*ml*	120*ml*	Not treated	40*ml*	80*ml*	120*ml*	Not treated	40*ml*	80*ml*	120*ml*
No. of plants	595	589	688	594	412	520	552	535	345	349	351	350
No. of diseased plants	329	138	138	118	137	293	122	139	61	26	27	6
Percentage diseased	55.3	23.2	23.5	19.9	33.3	56.3	22.2	26.0	17.7	7.4	7.7	1.7

him to pick up healthy ones only. The effects of chloropicrin sterilization for the control of this disease seem to differ according to the condition of the soil.

Experiment V.

The soil material for the experiment was collected from the seedbed where many cases of this disease had been seen in the spring of 1952, and was kept in this experiment station as in Experiment I. The infested soil was treated with chloropicrin (CP) and D-D as follows: (1) CP 120 ml, (2) same 240 ml, (3) D-D 40 ml, (4) same 80 ml, (5) same 120 ml, (6) CP: D-D 40 ml, (7) same 80 ml, (8) same 120 ml, (9) steam sterilization 90°C for 10 min, and (10) non-treatment were prepared for comparison. Mixing ratio of CP: D-D was 1: 2 volume. The seedbeds were electrically heated, and the seeds were sown on 15th September. All sterilizations were

TABLE 5. *Effects of chemical sterilization of the infested soil to development of the tobacco stunt disease*

(September to November, 1952)

Chemicals and quantity	(*ml*)	No. of plants	No of diseased plants	Percentage diseased
CP	120	429	23	5.4
CP	240	472	65	13.8
D-D	40	495	164	33.1
D-D	80	495	106	21.4
D-D	120	495	109	22.0
CP:D-D	40	336	135	40.2
CP:D-D	80	495	118	23.6
CP:D-D	120	494	94	19.0
Steam sterilization		495	0	0.0
Non-treatment		442	143	32.4

carried out two weeks in advance of seed sowing. There still remained fairly strong smell in D-D 80 ml section and 120 ml section at the time the surface soil was used in seedbed, but this had no effect on the germination and the growth of the seedlings. On 21st October the first case of the disease was found, and many cases followed around 28th of the same month. On 6th of November all the seedlings were rooted out for examination.

Table 5 shows that the two sections of chloropicrin treatments and CP : D-D 120 ml section proved some effects, but with others the effects were negligible. In steam-sterilization section, no disease case was seen verifying that this was the most effective method of sterilization.

Discussion

The results of sterilization experiments using chloropicrin, ethylene bromide, D-D, and the mixture of chloropicrin and D-D and by steam showed that steam sterilization was the most effective. Though the chloropicrin was most effective among the chemicals used, the results depended upon water content, temperature and other conditions, and they cannot be held reliable. Though the results of the sterilization were not favourable from the point of view of the number of disease cases, these grew in concentrated groups so that it was easier for the cultivator to pick up healthy seedlings only.

Summary

The writer studied the sterilization of the seedbed soil infested by the tobacco stunt disease with chloropicrin, D-D, ethylene bromide and steam. The steam sterilization at 90°C for 10 minutes was the most sure method of all to control the disease and chloropicrin was the best among the chemicals. Even when the infection took place on the sterilized soil, the disease occured in groups, and made it easy to pick out the healthy plants.

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EXPLANATION OF XI-PL. I

Fig. 1. Effects of sterilization of the infested soil with steam and chloropicrin to development of tobacco stunt disease.
Front row: steam-sterilization section.
Central row: chloropicrin 3 ml sterilization section.
Back row: chloropicrin 5 ml sterilization section.

Fig. 2. Effects of chloropicrin sterilization of the infested soil to development of the tobacco stunt disease.
Front row: non treatment section.
Back row: chloropicrin 1 ml sterilization section.



Fig. 1



Fig. 2

EXPLANATION OF XI-PL. II

Fig. 1. Effects of sterilization of the seedbed soil to development of the tobacco stunt disease.
Non-treatment section. Those marked with pieces of straw are the diseased seedlings.

Fig. 2. Effects of sterilization of the seedbed soil to development of the tobacco stunt disease.
Chloropicrin sterilization section. Those marked with pieces of straw are the diseased seedlings. Here they are seen in groups.



Fig. 1



Fig. 2

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